CHEMICALLY MEDIATED COMPETITION, HERBIVORY, AND THE STRUCTURE OF CORAL REEFS

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CHEMICALLY MEDIATED COMPETITION, HERBIVORY, AND THE STRUCTURE OF CORAL REEFS

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
aq	aqueous
C	Celsius, carbon
CCA	crustose coralline algae
CF-IRMS	continuous-flow isotope ratio mass spectrometry
cm	centimeter
d	day, days
DOC	dissolved organic carbon
E	east
g	gram
GT	Georgia Institute of Technology
h	hour, hours
H	hydrogen
+H	herbivores included
-H	herbivores excluded
HPLC	high-pressure liquid chromatography
HRMS	high-resolution mass spectrometry
HR-EMI-MS	high-resolution electrospray ionization mass spectrometry
HSD	honestly significant difference
K	potassium
Km	kilometer
L	liter
LC-MS	liquid chromatography-mass spectrometry
m	meter

MHz	megahertz
min	minute
mL	milliliter
MPA	marine protected area
m/z	mass to charge ratio
N	north, nitrogen
+N	nutrient enrichment
-N	no nutrient enrichment
Na	sodium
NMR	nuclear magnetic resonance
O	oxygen
P	phosphorus
PAM	pulse amplitude modulated
PSII	photosystem II
PVC	polyvinyl chloride
RDM	relative dominance model
S	south
SE	standard error
UV	ultraviolet
VPDB	Vienna Pee Dee Belemnite
vs.	versus
W	west
µL	microliter

LIST OF SYMBOLS

Y	effective quantum yield of photosystem II
ϕ_{PSII}	effective quantum yield of photosystem II
°	Degrees
%	percentage
‰	parts per thousand
δ	stable isotope

SUMMARY

Corals, the foundation species of tropical reefs, are in rapid global decline as a result of anthropogenic disturbance. On many reefs, losses of coral have coincided with the over-harvesting of reef herbivores, resulting in ecosystem phase-shifts from coral to macroalgal dominance. It is hypothesized that abundant macroalgae inhibit coral recovery and recruitment, thereby generating ecological feedback processes that reinforce phase-shifts to macroalgae and further diminish reef function. However the extent to which macroalgae directly outcompete coral, the mechanisms involved, and the species-specificity of algal-coral competition remains debated. Moreover the capacity for herbivores to prevent vs. reverse ecosystem phase-shifts to macroalgae, and the roles of herbivore diversity in such phenomen remain poorly understood.

Here I demonstrate using field experiments in the tropical Pacific (Fiji) and Caribbean Sea (Panama) that numerous macroalgae directly damage corals, and do so by transferring hydrophobic allelochemicals from algal to coral surfaces upon contact. Algal allelochemicals caused coral damage and/or mortality in ~75% of the 39 interactions studied, and allelopathic effects of algae on coral were generally localized to areas of direct contact. Coral sensitivity to algal allelopathy was highly species-specific. For two of the most damaging algae, I identified a subset of the molecules responsible for their allelopathic activity – these terpenoid molecules are the first identified allelochemicals that harm reef-building corals. Together these findings suggest that allelopathic algal-coral competition may commonly limit coral recovery on reefs lacking sufficient herbivory, and therefore reinforce ecosystem phase-shifts to macroalgae.

Here I also demonstrate with field experiments in Fiji that herbivory has the potential to both prevent and reverse coral reef phase-shifts to macroalgae. Herbivory inhibited the establishment of late-succession macroalgae on artificial substrates, both inside and outside of a marine reserve, during a 152 day field study; in contrast, herbivore exclusion resulted in macroalgal proliferation. Nutrient enrichment had little effect on algal growth, indicating top-down control of algal succession on coral reefs. In addition, herbivores consumed all macroalgae used in my algal-coral competition experiments when deployed in marine reserves. Macroalgal consumption could be attributed to feeding by just four fish species, and no macroalgae escaped predation because of complementary feeding among these herbivores; the most allelopathic algae were each consumed by different fish species. Complementary feeding among herbivores was driven largely by their differential tolerances to algal chemical defenses. Also, herbivores preventing the establishment of late-succession macroalgae (by scraping the substratum) differed fundamentally from those fishes removing established macroalgae. Thus, chemically mediated feeding complementarity makes herbivore diversity essential for preventing and reversing phase-shifts on coral reefs, and limiting the negative effects of algae on coral.

Finally, I demonstrate with field experiments in Fiji that competition and herbivory interact via macroalgal secondary chemistry. A chemically rich macroalga simultaneously induced allelochemicals and decreased anti-herbivore chemical defenses in response to competition with coral, resulting in an ecological trade-off for the alga – increased competitive ability but also increased susceptibility to predation in the field. This pattern for the alga was likely due to a trade-off in the production of different

molecules responsible for deterring competitors vs. consumers. If common among allelopathic algae, trade-offs in defensive chemistry may increase herbivore control of allelopathic algae or attenuate algal effects on coral, thus altering the role of allelopathic algae in ecological feedback processes limiting reef recovery.

Coral reefs provide ecosystem services critical to human societies, but are declining at alarming rates. These studies provide evidence that chemically mediated competitive and consumer-prey interactions play important roles in the underlying processes driving coral reef degradation and recovery. Moreover these studies suggest that competition and predation interact in complex ways to affect the ecology and evolution of chemical defense in macroalgae. By providing mechanistic-level insights into the processes controlling coral reef structure and function, such findings should be of broad interest to ecologists, and should also provide resource managers with critical information needed for effective management of these dynamic and diverse, but threatened ecosystems.

CHAPTER 1

CHEMICALLY RICH SEAWEEDS POISON CORALS WHEN NOT CONTROLLED BY HERBIVORES

Abstract

Coral reefs are in dramatic global decline, with seaweeds commonly replacing corals. It is unclear, however, whether seaweeds harm corals directly or colonize opportunistically following their decline and then suppress coral recruitment. In the Caribbean and tropical Pacific we show that, when protected from herbivores, ~40 to 70% of common seaweeds cause bleaching and death of coral tissue when in direct contact. For seaweeds that harmed coral tissues, their lipid-soluble extracts also produced rapid bleaching. Coral bleaching and mortality was limited to areas of direct contact with seaweeds or their extracts. These patterns suggest that allelopathic seaweed-coral interactions can be important on reefs lacking herbivore control of seaweeds, and that these interactions involve lipid-soluble metabolites transferred via direct contact. Seaweeds were rapidly consumed when placed on a Pacific reef protected from fishing but were left intact or consumed at slower rates on an adjacent fished reef, indicating that herbivory will suppress seaweeds and lower frequency of allelopathic damage to corals if reefs retain intact food webs. With continued removal of herbivores from coral reefs, seaweeds are becoming more common. This occurrence will lead to increasing frequency of seaweed-coral contacts, increasing allelopathic suppression of remaining corals, and continuing decline of reef corals.

Introduction

As foundation species, corals promote marine biodiversity, support a multitude of ecosystem functions, and provide goods and services critical to human societies (Done et al. 1996, Moberg and Folke 1999). However, coral reefs are in global decline, with reefs commonly converting from species-rich and topographically complex communities dominated by corals to species-poor and topographically simplified communities dominated by seaweeds (Hughes 1994, Gardner et al. 2003, Hughes et al. 2003, Bellwood et al. 2004, Carpenter et al. 2008). In the Caribbean, average cover of hard corals has declined by ~80% in the last three decades (Gardner et al. 2003) and more than 30% of the world's coral species face elevated risk of extinction (Carpenter et al. 2008). Monitoring (Hughes 1994), field experiments (Jompa and McCook 2002, Hughes et al. 2007, Burkepile and Hay 2008), and a meta-analysis (Burkepile and Hay 2006) all indicate that herbivory is critical in preventing seaweed replacement of corals. However, the extent to which seaweeds drive these shifts by outcompeting adult corals in the absence of herbivory, or proliferate only after coral mortality is triggered by other causes (such as disease or bleaching) is debated (Jackson et al. 2001, McCook et al. 2001, Aronson and Precht 2006, Mumby and Steneck 2008). To compound this uncertainty, studies addressing seaweed-coral competition have: (1) produced variable results, (2) rarely been conducted using numerous species-pairings, (3) varied in experimental techniques (complicating comparisons), and (4) sometimes been conducted in laboratory settings lacking ecologically realistic conditions (*e.g.*, flow and turbulence). Thus, the general importance of competition between established seaweeds and corals remains uncertain. An understanding of mechanisms determining the outcomes of seaweed-coral

interactions, and of how herbivory mediates these interactions, is needed if reefs are to be better managed, especially with the continuing harvest of reef herbivores (Jackson et al. 2001, Jackson 2008, Mumby and Steneck 2008).

The importance of physical vs. chemical mechanisms affecting seaweed-coral interactions is also unclear (McCook et al. 2001). Although smothering, shading, and abrasion by a limited number of seaweeds have been shown to negatively (McCook et al. 2001, River and Edmunds 2001, Jompa and McCook 2003a, Nugues and Bak 2006) or positively (Jompa and McCook 1998) affect coral, chemically mediated competition between adult corals and seaweeds has received limited attention. Numerous marine benthic organisms produce secondary metabolites that function to deter consumers or suppress competitors (Hay 2009). In field studies, seaweed secondary metabolites have been proposed as likely agents affecting coral mortality (Jompa and McCook 2003a, Jompa and McCook 2003b), but only one investigation has demonstrated seaweed allelopathy (against a soft coral) under ecologically realistic field conditions (de Nys et al. 1991). In contrast, laboratory-based studies of multiple seaweed-coral pairings suggest that release of seaweed primary metabolites (*i.e.,* sugars and carbohydrates) can indirectly trigger coral mortality through effects on coral-associated microbes (Smith et al. 2006). These laboratory-based effects have yet to be documented under field conditions, and a recent field study found no effect of nearby seaweeds on the severity and dynamics of a microbe-generated coral disease, suggesting that natural hydrodynamic conditions may limit the impacts of algal generated metabolites in the field (Vu et al. 2009). Thus the relative frequency, intensity, and general ecological effects of seaweed allelopathy against corals remain unknown, as do the chemical nature and

mechanisms of allelopathy between seaweeds and corals (*e.g.,* the activity of primary vs. secondary metabolites and the role of direct poisons vs. indirect effects on microbes).

Here we describe field experiments in the Caribbean and tropical Pacific designed to assess the outcomes of, and mechanisms involved in, seaweed-coral competition across multiple seaweed species and functional groups. Throughout these 20 d experiments, we monitored effects of seaweeds on coral bleaching/death and photosynthetic efficiency using photographic image analysis and *in situ* pulse amplitude modulated (PAM) fluorometry, respectively. To assess the most plausible mechanism for the patterns we observed in our experiments, we then tested the effect of lipid-soluble extracts from each seaweed on corals. These seaweeds were then transplanted onto reefs to determine how herbivory may mediate seaweed-coral competitive interactions by limiting seaweed abundance. Our results indicate that several common seaweeds produce lipid-soluble metabolites that damage corals when seaweeds and corals come into direct contact.

Materials and Methods

Experimental design and study organisms

We assessed the outcomes of, and mechanisms involved in, seaweed-coral competition by assaying the effects of common seaweeds in the Caribbean (Coco Point Reef, Bocas del Toro, Panama; 9º18.019'N, 82º16.350'W; June-July 2008) and tropical Pacific (Votua Reef, Viti Levu, Fiji; 18º13.049'S, 177º42.968'E; August-September 2008) on a common *Porites* species coral from each location. To create standardized units of seaweed-coral contact in the same environmental setting, we collected 6-8 cm branches of *Porites porites* (Panama) and *Porites cylindrica* (Fiji) and glued them

individually into small cement cones (Figure 1.1) with underwater epoxy (Emerkit, New Zealand). In each cement cone, we embedded 4 cm nails on opposite sides of the upper surface so that the ends of a three-strand rope holding a seaweed could be slipped over each nail head, to hold the seaweed in contact with the coral. We used representative-sized individuals of seaweeds that were common at each site. Intact, whole thalli were used to avoid stress compounds that might be released if seaweeds were clipped. Control corals received a rope without macroalgae. Our transplant procedures allowed for seaweed-coral contact representative of natural contacts observed in the field.

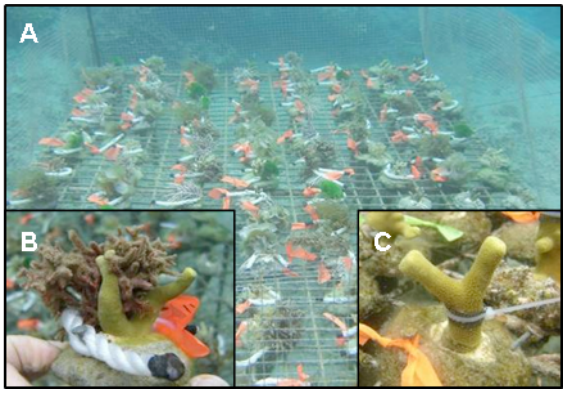

Figure 1.1: Experimental design. (A) A rack holding experimental corals in cones. (B) A coral replicate showing a seaweed transplanted against a coral. (C) A coral replicate wrapped with a gel containing the lipid-soluble extract of a seaweed.

We interspersed treatment and control replicates (n = 10-12 species^{-1}) haphazardly (15 cm apart in all directions) across five racks made of PVC (Panama) or welded metal (Fiji) frames holding metal mesh into which the bases of the cones could be placed (Figure 1.1). In Panama, the racks were secured on a coral-dominated reef, holding corals at 4 m depth. In Fiji, racks were secured on a coral-dominated reef flat, holding

corals at 1 m depth at low tide. *Porites* species were common around our racks at both sites. We caged racks with metal screening (1 cm^2 grid) to exclude large herbivores, and brushed cages every 2 d to remove fouling organisms. During routine maintenance, we visually noted bleaching of corals and replaced any seaweeds lost because of wave action (happened infrequently and only in Fiji). After 20 d, we assessed the effects of seaweed contact on coral tissue bleaching, relative to controls, using photographic surveys. Corals with bleaching were photographed with an underwater digital camera held perpendicular to the coral fragment. Using an in-frame scale, two-dimensional (2D) percent area bleaching of each replicate was quantified using ImageJ (v1.40, National Institutes of Health) photo analysis software. Because visual assessments of coral bleaching/mortality can be subjective (Fitt et al. 2001), we also quantified the effects of seaweed contact on coral bleaching after 20 d using *in situ* PAM fluorometry. Measurements were taken at the most damaged location of seaweed-coral contact and at the same height on the opposite side of the coral branch. These latter measurements assessed effects on coral tissues only millimeters away from affected tissues, but not in direct physical contact with seaweeds. We sampled control corals in the same manner (at a similar height on the side with the control rope and on the side opposite the rope).

In these field experiments, we used the corals *P. porites* (Caribbean Panama) and *P. cylindrica* (Fiji) because this is a pan-tropical genus common to both sites and used in other investigations of coral-seaweed competition (River and Edmunds 2001, Jompa and McCook 2002, Jompa and McCook 2003a, Jompa and McCook 2003b, Nugues and Bak 2006). The seaweeds we used were (1) common-to-abundant on these Poritid-dominated

reefs, (2) observed in contact with corals, and (3) representative of a range of taxonomic and morphological forms.

Algal mimic study

We also tested possible effects of abrasion and shading alone using inert algal mimics. We constructed a foliose mimic of *Padina* by cutting opaque fronds from black plastic bags and grouping them with cable-tie "holdfasts" (Figure 1.4C); a filamentous mimic of *Chlorodesmis* was made by cutting 60 loops of Dacron line (White River Fly Shop Magibraid Flyline Backing) into filaments and grouping them with a cable-tie "holdfast" (Figure 1.4D). Algal mimics ($n = 10$ treatment^{-1}) were then inserted into segments of three-strand rope and attached to fragments of *P. cylindrica* on racks at Votua Reef, Fiji (see experimental design, above). Control corals ($n = 10$) were also deployed with rope segments lacking an algal mimic (Figure 1.4E). Effects of algal mimics or controls on coral bleaching were assessed after 16 d using photographic surveys and *in situ* PAM fluorometry as described above (see Figure 1.4A & B).

Allelochemical bioassays

We exhaustively extracted whole tissues (20 mL displacement volume) of each alga with 100% methanol, filtered the extract, and removed the solvent by rotary evaporation. We re-suspended each extract in 15 mL of ethyl acetate, added it to 200 mL of water and an additional 200 mL of ethyl acetate in a 1 L separatory funnel, and obtained the lipid-soluble fractions of each alga by collection of the ethyl acetate layer. This was repeated three times for each sample to assure efficient partitioning. Each lipid-soluble extract was dried by rotary evaporation and stored at -5^{o}C for 2-3 d until bioassay preparation.

For bioassays, we re-suspended lipid-soluble extracts in 1 mL of methanol and added them at appropriate volumetric concentration to Phytagel (Sigma-Aldrich) bioassay squares (1 cm^2) that were formed on window screen (modified methods of Thacker et al. 1998). Control gels were created in the same manner, including the addition of methanol, but lacking seaweed extract. Gels were refrigerated for 7-10 h until deployed in the field. For deployment, a square (n = 10 $treatment^{-1}$) was wrapped around a coral branch and held in place by a cable tie (Figure 1.1C). After 24 h, we removed each strip and took a PAM fluorometry reading under the center of each treatment and control square.

We also extracted lipophilic metabolites from the surfaces of four Fijian seaweeds (three allelopathic, one not) using the "hexane dip" method (de Nys et al. 1998) to test if allelopathic metabolites were on seaweed surfaces at ecologically relevant concentrations that could produce the allelopathic effects we observed in our whole-tissue allelochemical bioassays. Samples (20 mL displacement volume) were collected from the field, excess water was removed in a salad spinner, and the alga was extracted with 100% hexanes for 30 s while vortexing (de Nys et al. 1998). We then dried each lipophilic extract by rotary evaporation, re-suspended them in 500 µL of hexanes, and added them at natural volumetric concentration to Phytagel squares as described above. Controls were created in the same manner, including the addition of hexanes, but lacking seaweed extract. Treatment and control gel squares (n = 10 $extract^{-1}$) were deployed and assayed in the same manner as the whole-tissue allelochemical bioassays.

PAM fluorometry

PAM fluorometry was used *in situ* to assess the effects of seaweeds and their extracts on coral health (effective quantum yield). PAM fluorometry provides a more rigorous and quantifiable measure of coral bleaching compared to visual assessments alone (Fitt et al. 2001 and references within, Smith et al. 2006). Effective quantum yield is a measure (unitless, ranging from 0.0-1.0) of the efficiency of photosystem II (PSII) within light-adapted photosynthetic organisms (*i.e.*, under ambient field conditions), which corresponds with the health of the organism (Fitt et al. 2001, Smith et al. 2006). Values for healthy corals typically range from 0.5 to 0.7 (*i.e.*, maximum potential quantum yield), depending on coral species and depth (Fitt et al. 2001). Values of ~0.0 to 0.2 are indicative of severe bleaching and mortality (Smith et al. 2006).

We took all PAM fluorometry readings between 0900-1400 h, and interspersed readings for all treatments and controls in time so that readings for a treatment would not be confounded by time (and associated variance in light or temperature). We observed low within-treatment variance (Figure 1.2) for all of our treatments and controls, indicating minimal variance due to time of sampling.

Seaweed palatability assays

To assess how herbivory might impact seaweeds and thus the probability of seaweed-coral contacts, we conducted field feeding assays in both September 2008 and August 2009 using the seaweed species from our 20 d field competition study in Fiji. *Liagora* sp. was not included in 2009 assays because of its scarcity at that time. We conducted these studies in Fiji because of close proximity of protected and fished reefs (~300 m apart), which allowed us to assess the survivorship of each seaweed species in the presence and absence of a diverse herbivore guild (Simpson 2009). We collected

each seaweed species from the same location that we collected seaweeds for our competition study and chemical extractions. Each year, standardized thalli of each seaweed (8-9 cm in height) were inserted 3-5 cm apart on a 60 cm length of three-strand rope, and deployed at intervals of ~5 m across a protected and fished reef ($n = 20$ site^{-1}, methods of Hay 1984). After 24 h, we visually scored seaweeds on each rope *in situ* as 0, 25, 50, 75, or 100% consumed, based on changes in seaweed height. Ropes at both sites were scored by the same individual to prevent observer bias. Caged controls were not deployed, as both sites within each location had similar topography and hydrodynamic conditions, and seaweeds that were 100% consumed still had basal remnants in the rope that showed grazing marks from fishes. If we pulled seaweeds from ropes (as a wave might), the entire seaweed thallus pulled free rather than breaking off at the base; thus, we could detect no evidence of loss to processes other than fish feeding.

Benthic surveys

We quantified benthic cover of macroalgae and hard corals in Votua Village's marine protected area (MPA) and 300 m west of the MPA by running 30 m transect surveys ($n = 10$ site^{-1}). In the middle of each site, we deployed the first transect according to a randomly generated compass bearing, and ran subsequent transects parallel to this initial transect. Perpendicular distances between each transect were randomly assigned. Macroalgae and hard corals were scored (presence/absence) at 1 m intervals along each transect to determine percent cover.

Statistical analysis

Data from our field competition and allelochemical bioassays violated parametric assumptions, so we evaluated them with Kruskal-Wallis Analysis of Variance (ANOVA)

on ranks. When some replicates lost seaweeds or were missed during final scoring, we randomly excluded replicates from other treatments (~1-2) to equalize sample sizes and allow for more powerful post-hoc tests that require balanced sample sizes. The algal mimic assay results were analyzed by one factor ANOVA. Differences among subgroups were analyzed for all ANOVAs using Student-Newman-Kuels post-hoc tests. Herbivory assays produced ordinal data, so they were analyzed by Mann-Whitney U tests (Zar 2010). We analyzed transect data using a t test (for hard coral cover) and a Mann-Whitney U test (for macroalgal cover).

Results

Seaweed effects on corals

When the coral *Porites porites* (Panama) was placed in direct contact with seven common seaweeds for 20 d, *Ochtodes secundaramea*, *Dictyota bartayresiana*, *Lobophora variegata*, *Halimeda opuntia*, and *Amphiroa fragillisima* caused significant bleaching relative to controls ($p < 0.001$, n = 9; Figure 1.2A), while *Padina perindusiata* or *Sargassum* sp. did not. Because visual assessments of coral bleaching and mortality can be subjective (Fitt et al. 2001), we also analyzed the effects of seaweeds on coral photosynthetic efficiency (effective quantum yield) using *in situ* PAM fluorometry, a method that quantifies coral health in response to environmental stressors (Fitt et al. 2001, Smith et al. 2006). Symbiont photosynthetic efficiency was highly correlated with bleaching ($r = -0.96$, $p < 0.001$; Figure 1.3). Paralleling patterns of bleaching and mortality, *O. secundaramea*, *D. bartayresiana*, *L. variegata*, *H. opuntia*, and *A. fragillisima* suppressed photosynthetic efficiency of *P. porites* by 52-90% relative to controls ($p < 0.001$, n = 9; Figure 1.2C), while *P. perindusiata* and *Sargassum* sp. had no

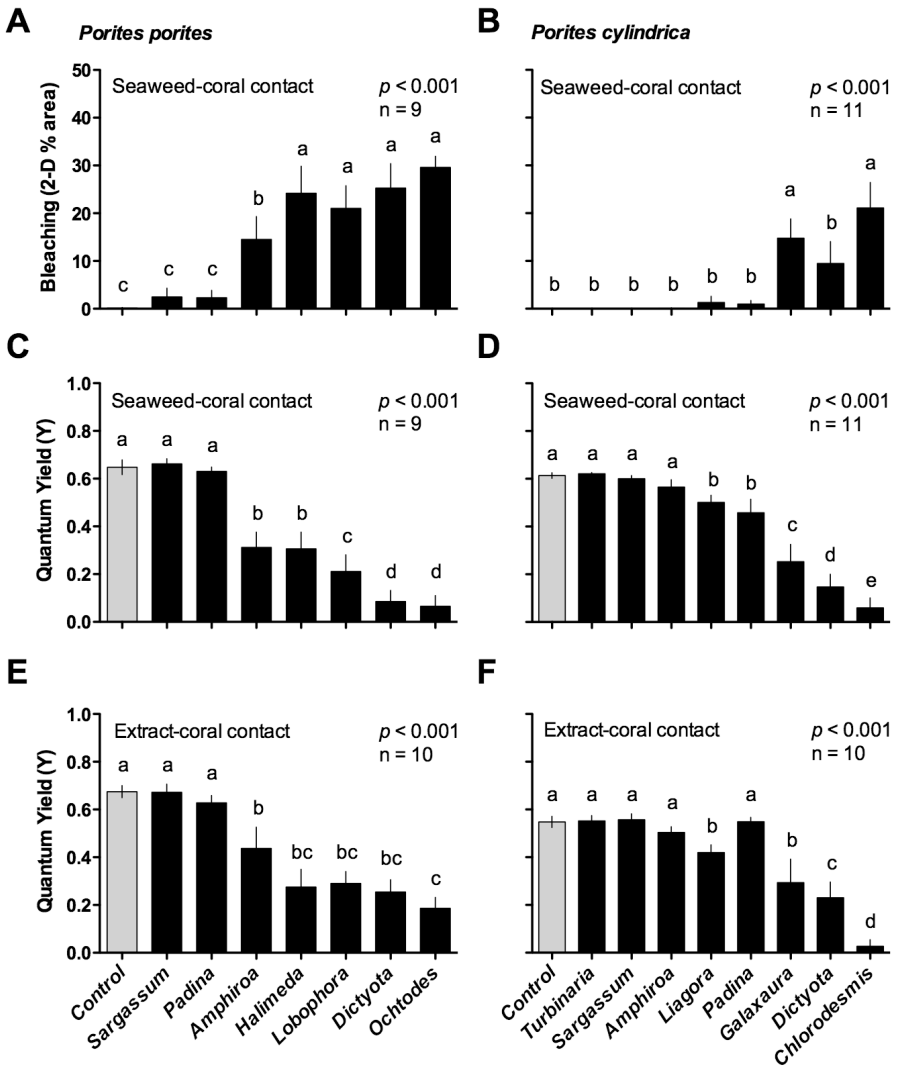

Figure 1.2: Effects of seaweeds and their extracts on coral health. (A & B) Visual coral tissue bleaching (2-D % area; mean ± SE) and (C-F) photosynthetic efficiency (Y; mean ± SE) of the corals *Porites porites* in Panama and *Porites cylindrica* in Fiji when in contact with seaweeds for 20 d (A-D, n = 9-11), or in contact with gel squares containing lipid-soluble extracts from the same seaweeds for 24 h (E & F, n = 10). Analyzed by Kruskal-Wallis Analysis of Variance on ranks. Letters indicate homogeneous subgroups by post-hoc Student-Newman-Kuels tests.

effects. Corals in contact with the most harmful seaweeds had effective quantum yields indicative of severe bleaching and mortality (Smith et al. 2006 and references within). Neither visual bleaching, nor suppression of photosynthetic efficiency occurred on thesides of corals away from seaweed-coral contact (5-10 mm from seaweed contact; p = 0.358, n = 9). Thus, seaweeds damaged corals only in areas of direct contact.

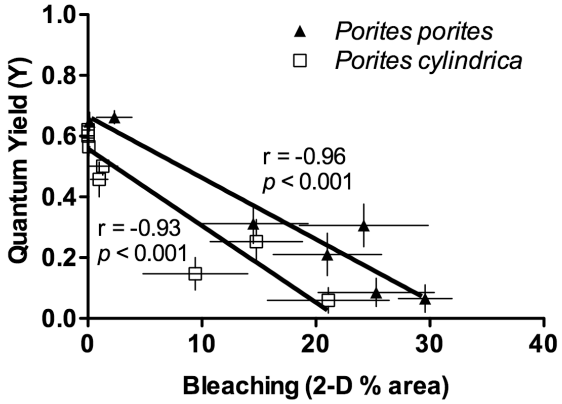

Figure 1.3: Linear correlation between coral bleaching and photosynthetic efficiency for both corals. Values determined for corals in direct contact with seaweeds for 20 d (mean ± SE; n = 9-11 seaweed-coral treatment^{-1}). Analyzed by Pearson's correlation coefficients.

Results for tests with *Porites cylindrica* (Fiji) were similar to those from Panama. When *P. cylindrica* was in contact with eight common seaweeds for 20 d, *Chlorodesmis fastigiata* and *Galaxaura filamentosa* caused significant visual bleaching relative to controls (p < 0.001, n = 11; Figure 1.2B), while *Padina boryana*, *Liagora* sp.*, Amphiroa crassa, Sargassum polycystum*, and *Turbinaria conoides* caused no significant visual coral bleaching. *Dictyota bartayresiana* caused appreciable visual bleaching, but did not

statistically differ from controls by post-hoc analysis. *P. cylindrica* bleaching correlated with photosynthetic efficiency ($r = -0.93$, $p < 0.001$; Figure 1.3), and corals in contact with harmful seaweeds had effective quantum yields indicative of severe bleaching/mortality ($p < 0.001$, $n = 11$; Figure 1.2D). In contrast, *S. polycystum*, *T. conoides*, and *A. crassa* had no effect on coral bleaching or photosynthetic efficiency. The seaweeds *P. boryana* and *Liagora* sp. caused slight, but significant suppression of *P. cylindrica* photosynthetic efficiency (Figure 1.2D) relative to controls, despite not generating significant visual bleaching (Figure 1.2B). Contact with these seaweeds produced stress unrecognizable by visual assessments alone. As with *P. porites* in Panama, neither significant visual bleaching, nor suppression of photosynthetic efficiency occurred on the far sides of *P. cylindrica* away from seaweed contact ($p = 0.794$, $n = 11$). Thus, Fijian seaweeds also caused bleaching only in areas of direct contact.

Seaweeds could have affected corals via abrasion, shading, or lipid-soluble allelopathic compounds transferred by direct contact rather than via dissolution into the water. When inert models designed to mimic the shading and abrasion of bladed species such as *Padina* and filamentous species such as *Chlorodesmis* were placed in direct contact with *P. cylindrica* for 16 d in the field, *Padina* mimics and *Chlorodesmis* mimics caused no bleaching or effects on photosynthetic efficiency relative to controls (Figure 1.4). Thus, physical effects of abrasion and shading were not detectable in our experiment.

Extract effects on corals

When lipid-soluble extracts from each Panamanian seaweed were embedded at natural volumetric concentration in Phytagel squares and placed in direct contact with *P.*

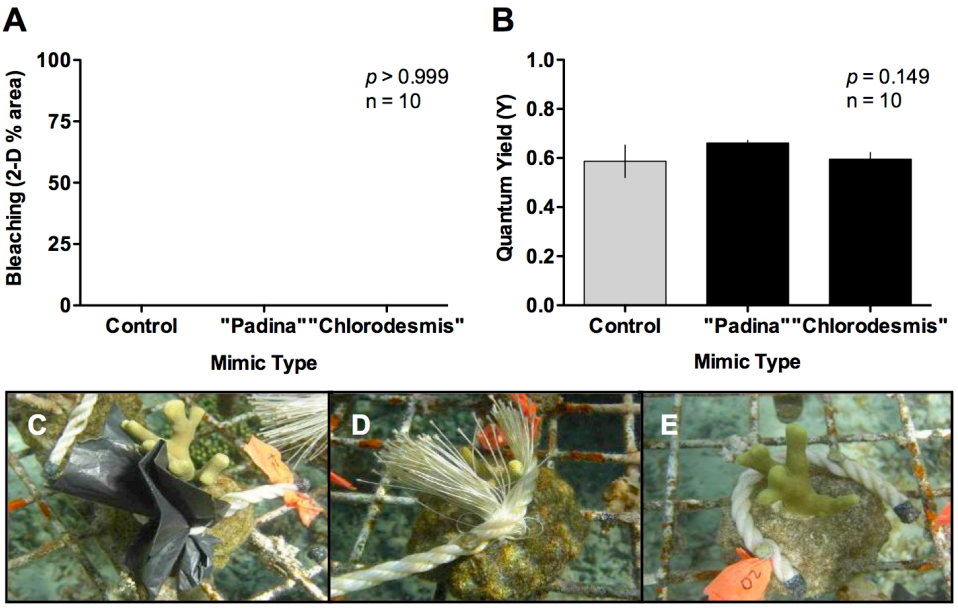

Figure 1.4: Effects of seaweed models on coral health. (A) Visual coral tissue bleaching (2-D % area; mean ± SE) and (B) photosynthetic efficiency (Y; mean ± SE) of the coral *Porites cylindrica* when in direct contact for 16 d with seaweed models designed to mimic (C) *Padina* and (D) *Chlorodesmis*, relative to (E) controls (n = 10). Analyzed by one factor Analysis of Variance.

porites for 24 h in the field (Figure 1.1C), effects of extracts paralleled effects of direct seaweed contact; *O. secundaramea, D. bartayresiana, L. variegata, H. opuntia,* and *A. fragillisima* caused significant coral bleaching and suppression of photosynthetic efficiency in assays using both intact seaweeds ($p < 0.001$, n = 9; Figure 1.2C) and chemical extracts ($p < 0.001$, n = 10; Figure 1.2E). *Padina perindusiata* and *Sargassum* sp. caused no significant bleaching in either assay.

In Fiji, extracts from *C. fastigiata*, *D. bartayresiana*, *G. filamentosa,* and *Liagora* sp. caused bleaching and suppression of photosynthetic efficiency of *P. cylindrica* relative to controls ($p < 0.001$, n = 10; Figure 1.2F); extracts of *P. boryana*, *A. crassa, S. polycystum*, and *T. conoides* did not. With the exception of *P. boryana*, effects of Fijian seaweeds in assays using intact plants (Figure 1.2D) were mirrored by effects of lipid-soluble extracts (Figure 1.2F). *Padina* was unusual in that it suppressed effective quantum yield by 25% in whole-seaweed assays, but its extract produced no rapid allelopathic effect. It is possible that its extract acts slowly, or that the modest effect of *P. boryana* that we detected in our 20 d whole-plant assay was a mild effect of shading or abrasion.

The effects of extracts were produced by extracting entire algal thalli. This could be unrealistic if the allelopathic metabolites we detected were *in*, but not *on*, seaweeds where they could be transferred to corals. When lipophilic molecules were extracted from only the surfaces of four Fijian seaweeds (methods of de Nys et al. 1998), incorporated into Phytagel squares, and placed in contact with *P. cylindrica* for 24 h in the field, surface extracts of *C. fastigiata*, *D. bartayresiana*, and *G. filamentosa* caused bleaching and suppression of photosynthetic efficiency relative to controls ($p < 0.001$, n =

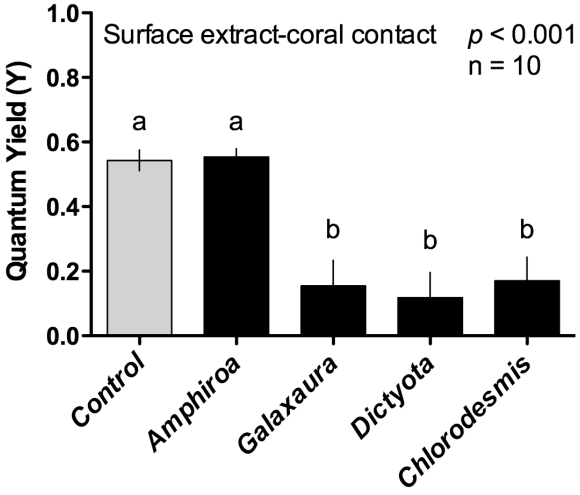

Figure 1.5: Effects of seaweed surface extracts on coral health. Photosynthetic efficiency (Y; mean ± SE) of *Porites cylindrica* in direct contact for 24 h with gel squares containing lipid-soluble extracts from the surfaces of seaweeds (n = 10). Analyzed as in Figure 1.2.

10; Figure 1.5). In contrast, surface extracts of *A. crassa*, which had no effect in whole-plant assays, had no significant effects. Thus, the effects of surface extracts, of whole-plant extracts, and of assays using intact plants all indicate that lipid-soluble allelopathic metabolites occur on algal surfaces and damage adjacent corals following direct contact.

Herbivore effects on seaweeds

Our experiments were performed in a marine protected area (MPA) of Votua Village's reef flat, Fiji. In this MPA, coral cover is high (57 ± 3%; mean ± SE) and macroalgal cover is low (3 ± 1%). In contrast, the adjacent reef flat 300 m west of the MPA is heavily fished and has low coral (3 ± 2%) and high macroalgal cover (47 ± 5%). Cover of both corals and macroalgae differ between sites ($p < 0.001$ and $p < 0.001$ respectively, n = 10).

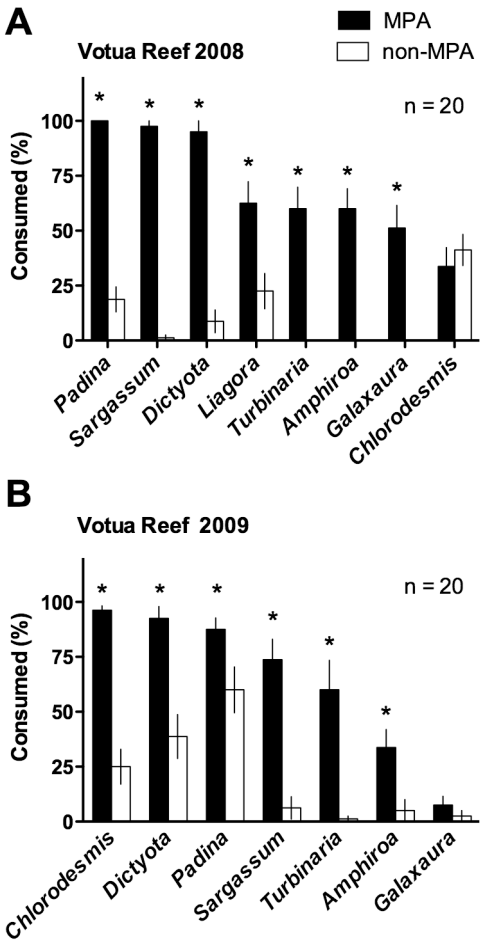

Figure 1.6: Consumption of seaweeds in a marine protected area (MPA) and on an adjacent fished reef. Seaweeds consumed (%; mean ± SE) by herbivores during a 24 h feeding assay on a protected (n = 20) and fished (n = 20) reef (~300 m apart) in (A) 2008 and (B) 2009. Stars indicate differences in the consumption of a seaweed between reefs, within a year, by Mann-Whitney U tests.

In 2008, when we transplanted all macroalgae used in our caged competition study into both sites, losses over 24 h in the MPA were 40-100% for all species; losses in the fished area were 0-40% (Figure 1.6A). For all species but *C. fastigiata*, rates of grazing in the MPA were significantly higher than on the fished reef flat. When repeated in 2009, trends were similar. Six of the seven species were consumed significantly more in the MPA; *G. filamentosa* was minimally consumed in both sites (Figure 1.6B).

Discussion

In both the Caribbean and tropical Pacific, contact of seaweeds with *Porites* corals commonly caused bleaching, lowered photosynthetic efficiency, and in several cases death of coral tissues in areas of contact. These patterns were reproduced when corals were in contact with only the lipophilic extracts of these seaweeds, suggesting that seaweeds damaged corals via chemical mechanisms. Our inert algal mimics produced no detectable effects on corals, also indicating chemical instead of physical effects.

In Panama, five of seven seaweeds (71%) caused bleaching of *Porites porites*; in Fiji, three of eight species (38%) caused bleaching of *Porites cylindrica*. We commonly observed these *Porites* spp. in contact with seaweeds at our field sites, suggesting that this genus may be relatively tolerant of contacts, potentially making these data conservative relative to other corals. As reefs are increasingly depleted of herbivores that suppress seaweeds (Hughes 1994, Jackson et al. 2001, Bellwood et al. 2004, Jackson 2008, Mumby and Steneck 2008), coral-seaweed contacts will increase in frequency, enhancing the damage that corals may experience from allelopathic seaweeds. Thus, in addition to suppressing recruitment and growth of new corals (Birrell et al. 2008a), several common seaweeds (Figure 1.2) can damage adult corals using allelochemicals.

To date, the few demonstrated allelopathic interactions among reef species all rely on transfer of metabolites via direct contact rather than via transmission through the water (de Nys et al. 1991, Thacker et al. 1998, Kubanek et al. 2002), suggesting that allelopathic metabolites are lipid- rather than water-soluble and that their effects are generated by contact rather than proximity alone. The primacy of lipophilic molecules as allelopathic agents makes evolutionary and energetic sense given the ocean's potential to dilute and advect water-soluble metabolites.

Although the activity of lipid-soluble extracts matched patterns from intact algae in 93% of the interactions we investigated, physical mechanisms such as shading or abrasion may be important for some seaweed-coral interactions, or for interactions lasting longer than 20 d. However, patterns of coral bleaching did not correlate well with seaweed structure that should affect abrasion; seaweeds that caused bleaching commonly had a soft, non-abrasive thallus (*e.g., Ochtodes, Chlorodesmis, Dictyota*), while tougher, more abrasive species such as *Turbinaria* and *Sargassum* did not damage corals. Additionally, some of the most chemically active seaweeds in Fiji (*Chlorodesmis* and *Dictyota*) produced obvious bleached areas after only 2 d of contact; algal mimics designed to cause abrasion and shading had no effect after 16 d (Figure 1.4). Moreover, assays using extracts from algal surfaces alone demonstrated that allelopathic metabolites are at sufficient surface concentrations to damage corals. Recent studies show that multiple seaweeds deploy secondary metabolites on their surfaces where they could play allelopathic roles (Nylund et al. 2007, Lane et al. 2009).

Although numerous seaweeds associated with degraded reefs (*e.g., Lobophora, Halimeda, Dictyota*, *Amphiroa*) bleached corals in our study, a few seaweeds that are

common following herbivore removal (*Sargassum, Turbinaria*, *Padina*) did not rapidly damage corals. To avoid confounding density and species effects, we deployed one seaweed thallus per replicate in our field experiments. It is possible that our results are conservative and that seaweeds such as *Sargassum*, *Padina*, and *Turbinaria* may need to grow in greater abundance or for greater lengths of time to produce impacts on coral health. Indeed, some studies have detected effects of *Sargassum* on *Porites* growth (via abrasion) in < 20 d using greater seaweed abundance in treatments (River and Edmund 2001), and have found large stands of *Sargassum* to be associated with increased *Porites* mortality and decreased coral recruitment within experimental fish exclosures over longer time periods (Hughes et al. 2007).

Seaweeds like *Dictyota* that both bloom on overfished reefs (Lirman and Biber 2000) and are strongly allelopathic (Figure 1.2) may be especially damaging to corals, although *Dictyota* species appear variable in their allelopathic activities (Box and Mumby 2007). Fortunately, other strongly allelopathic species like *Chlorodesmis, Galaxaura,* and *Ochtodes* rarely become abundant on reefs. However, our observations of fishes feeding on our algal transplants in Fiji indicated that a single herbivorous fish (*Siganus argenteus*) was responsible for all grazing on *C. fastigiata* (see also Paul et al. 1990), suggesting that suppression of even a single herbivore species in this diverse community could elevate risk of coral degradation via algal allelopathy.

Recent studies found that water-soluble leechates from seaweeds caused rapid coral mortality in the laboratory via effects on coral-associated microbes and suggested this was due to microbial stimulation by dissolved organic carbon (DOC) (Smith et al. 2006). Our results were consistent with seaweeds damaging corals via lipid-soluble

allelochemicals transferred during contact; we detected no near-contact effects (*i.e.*, on opposite side of corals just millimeters away from seaweed contact) that might be expected if water-soluble primary metabolites were damaging corals. Whether lipid-soluble secondary metabolites act as direct coral poisons or via effects on coral-associated microbes (Nugues et al. 2004, Smith et al. 2006) was not tested, but the lack of an impact that spread beyond areas of direct contact may be most parsimoniously explained as a direct allelochemical effect. Regardless of mode of action, direct contact between corals and several seaweeds produced allelopathic interactions that damaged corals. Seaweed primary (dissolved organic carbon) and secondary metabolites might also interact synergistically to harm corals, with the importance of differing metabolites varying under different environmental conditions.

We conducted our competition studies using a caged design that excluded herbivores, simulating modern reef conditions where herbivorous fishes have been over-harvested (Jackson et al. 2001, Jackson 2008). When seaweeds from our Fijian competition study were placed in the field within a MPA and 300 m away in a fished area, most seaweeds were rapidly consumed in the MPA (Figure 1.6) hosting a diverse herbivore guild (Simpson 2009), but consumed much more slowly or at undetectable rates on the adjacent reef subject to fishing. Several of the seaweeds consumed in our feeding assays demonstrated potent allelopathic activity against corals, and are known to be rich in secondary metabolites that deter some reef herbivores (*e.g., Dictyota, Chlorodesmis, Ochtodes, Halimeda*). Thus, even modest harvesting of those fishes that consume chemically rich seaweeds (Schupp and Paul 1994, Burkepile and Hay 2008) could lead to increases in some of the most chemically damaging seaweeds and to

increasing allelopathic impacts on reef corals. Moreover, these findings indicate that feeding complementarity (Burkepile and Hay 2008) and high grazing rates typical of healthy, less-fished reefs (Hughes 1994, Hughes et al. 2007, Jackson 2008; Mumby and Steneck 2008) should lessen allelopathic damage to corals by limiting seaweed abundance, and thus seaweed-coral contacts.

Our results show that numerous seaweeds can damage corals via allelochemicals. Such chemical effects could produce the suppression of coral fecundity and recruitment noted by previous investigators (Birrell et al. 2008a, Birrell et al. 2008b), and could produce negative feedbacks making reef recovery less likely as seaweed abundance increases (Mumby and Steneck 2008). Chemically mediated seaweed-coral competition may limit recovery of present day coral reefs regardless of the factors causing initial coral decline. This will be especially true where local factors (*e.g.*, overfishing) interact with global factors (*e.g.*, climate change) to change reef community structure over large spatial scales that limit the ability of herbivores to control seaweed abundance. Information on which seaweeds damage corals and which herbivore species best limit these seaweeds may prove useful in better managing reef resilience to facilitate recovery (Bellwood et al. 2004, Bellwood et al. 2006, Hughes et al. 2007, Burkepile and Hay 2008).

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CHAPTER 2

MACROALGAL TERPENES FUNCTION AS ALLELOPATHIC AGENTS AGAINST REEF CORALS

Abstract

During recent decades, many tropical reefs have transitioned from coral to macroalgal dominance. These community shifts increase the frequency of algal-coral interactions and may suppress coral recovery following both anthropogenic and natural disturbance. However, the extent to which macroalgae damage corals directly, the mechanisms involved, and the species specificity of algal-coral interactions remain uncertain. Here we conducted field experiments demonstrating that numerous macroalgae directly damage corals by transfer of hydrophobic allelochemicals present on algal surfaces. These hydrophobic compounds caused bleaching, decreased photosynthesis, and occasionally death of corals in 79% of the 24 interactions assayed (three corals x eight algae). Coral damage generally was limited to sites of algal contact, but algae were unaffected by contact with corals. Artificial mimics for shading and abrasion produced no impact on corals, and effects of hydrophobic surface extracts from macroalgae paralleled effects of whole algae; both findings suggest that localized effects were generated by allelochemical rather than physical mechanisms. Rankings of macroalgae from most to least allelopathic were similar across the three coral genera tested. However, corals varied markedly in susceptibility to allelopathic algae, with globally declining corals such as *Acropora* more strongly affected. Bioassay-guided fractionation of extracts from two allelopathic algae led to identification of two loliolide

derivatives from the red alga *Galaxaura filamentosa* and two acetylated diterpenes from the green alga *Chlorodesmis fastigiata* as potent allelochemicals. Our results highlight a newly demonstrated but potentially widespread competitive mechanism to help explain the lack of coral recovery on many present day reefs.

Introduction

Corals are structurally complex foundation species that generate and maintain tropical reef biodiversity. However, the direct and interactive effects of climate-induced coral bleaching (Hoegh-Guldberg et al. 2007, Baker et al. 2008), ocean acidification (Baker et al. 2008, De'ath et al. 2009), coral disease (Bruno et al. 2007), coastal overfishing and eutrophication (Jackson et al. 2001, Pandolfi et al. 2003, Hughes et al. 2007, Carilli et al. 2009) have led to coral decline over wide areas. On many reefs, dramatic declines in coral cover have co-occurred with significant increases in fleshy macroalgae (Hughes 1994, Bruno et al. 2009, Hughes et al. 2010). Once established, macroalgae can inhibit coral recruitment and decrease herbivore grazing, producing negative feedbacks that reinforce phase-shifts and further diminish reef function (Birrell et al. 2008, Mumby and Steneck 2008, Hoey and Bellwood 2011). Thus, local (*e.g.*, overfishing) and global (*e.g.*, climate) stresses may interact in complex ways to suppress coral cover, promote algal proliferation, and compromise reef resilience; such complexities provide both challenges and opportunities for managing these dynamic ecosystems (Mumby and Steneck 2008, Hughes et al. 2010).

As corals decline and macroalgae proliferate, the frequency of algal-coral interactions will increase, potentially affecting the survivorship, growth, and reproduction of remnant adult corals and new coral recruits (Birrell et al. 2008, Mumby and Steneck

2008). However, the consequences of and mechanisms driving most algal-coral interactions remain poorly understood. Recent field studies suggest that macroalgae may damage corals by (1) shading and abrasion (Box and Mumby 2007), (2) vectoring of coral disease (Nugues et al. 2004), (3) release of water-soluble compounds that stimulate harmful, coral-associated microbes (Smith et al. 2006), or (4) transfer of hydrophobic allelochemicals by direct contact (Rasher and Hay 2010). However, for most of these studies, it is unclear whether the findings are particular to the macroalgal and coral species tested, or are common to algal-coral interactions in general and could thus transform the way ecologists and resource managers view processes driving phase-shifts on coral reefs. Despite recent studies demonstrating the potential importance of chemically mediated algal-coral competition (Smith et al. 2006, Rasher and Hay 2010, Diaz-Pulido et al. 2010) and its increasing impact as a result of ocean acidification (Diaz-Pulido et al. 2011), no algal compounds mediating these interactions have been identified.

Here we assessed the role of seaweed allelopathy in algal-coral interactions across three abundant shallow-water corals contacting eight common macroalgae. We monitored effects of macroalgal contact on coral bleaching using photographic image analysis and *in situ* pulse amplitude modulated (PAM) fluorometry. To examine the probable mechanism producing the field patterns we observed, we assessed the effects of hydrophobic chemistry from whole-algal extracts and from surface-only extracts of chemically active macroalgae on each coral. For two of the most damaging macroalgae, we used a bioassay-guided fractionation approach to isolate and identify four surface-associated compounds that were allelopathic to corals. Our results indicate that

numerous macroalgae harm a diverse array of corals using allelochemicals and that harmful macroalgae contain multiple hydrophobic compounds that fulfill this allelopathic role.

Materials and Methods

Experimental design and study organisms

In July 2008, we collected branches of the corals *Montipora digitata*, *Acropora millepora*, and *Pocillopora damicornis* from colonies on Votua Reef, Viti Levu, Fiji (18º13.049'S, 177º42.968'E) and epoxied them (Emerkit) individually into small cement cones. Corals then were transplanted onto reef flat coral racks (1 m deep at low tide) and given 7 weeks to acclimate before experiments. We embedded 4 cm nails on opposite sides of the surface of each cone so that a three-stranded rope holding an alga could be slipped over each nail head to hold the alga in contact with the coral. Control corals received a rope but lacked macroalgae. Control algae were deployed in ropes on cement cones but lacked a coral. In our algal-coral contact experiments we used representative-sized individuals of macroalgae that (1) were common around our site, (2) were observed in contact with corals, (3) represented a range of taxonomic and morphological forms, and (4) were used in a previous study with *Porites cylindrica* from this site with the same experimental design (Rasher and Hay 2010), thus making possible contrasts across four coral genera. Whole thalli were used to avoid stress compounds that might be released from clipped macroalgae. These procedures produced algal-coral contacts representative of interactions we observed in the field.

To simulate the effects of macroalgae on remnant adult coral colonies or juvenile corals recruiting to adult populations, we utilized 6-8 cm length individuals of each coral

species in our experiments. We chose the branching corals *M. digitata*, *A. millepora*, and *P. damicornis* because they (1) are common at our study sites, (2) are three of the dominant corals on reef flats in Fiji, (3) could be fragmented with minimal damage to the host colony, and (4) possess a range of life-history traits, including brooding vs. broadcast reproduction.

We interspersed treatment and control replicates (n = 12 species^{-1}) haphazardly (15 cm apart in all directions) across five racks of metal mesh into which the bases of the cones could be placed. Racks were secured 3-4 m apart on a coral-dominated reef flat in Votua Village's no-take marine reserve. At low tide, corals in these racks were at ~1 m depth. We caged racks with metal screen (1 cm^2 grid) to exclude large herbivores and brushed cages every 2 d to remove fouling organisms. During routine maintenance, we replaced macroalgae lost to wave action (an infrequent occurrence). After 2, 10, and 20 d, we assessed the effects of algal contact on coral bleaching, relative to controls. Any bleaching on corals was photographed, and the two-dimensional (2D) percent area bleached in each replicate was quantified using ImageJ (v1.40, National Institutes of Health) photo analysis software. Because visual assessments can be subjective (Fitt et al. 2001, Smith et al. 2006, Pawlik et al. 2007, Rasher and Hay 2010), we also assessed coral bleaching and algal photosynthesis using *in situ* PAM fluorometry (Walz). Fluorometry measurements were taken on treatment corals at the most damaged location of algal-coral contact. To assess effects on coral tissues only millimeters away from affected tissues but not in direct contact with macroalgae, we also sampled at the same height on the opposite side of the coral branch. Fluorometry measurements of treatment algae were

taken at the site of greatest coral contact. Control corals and algae were sampled in the same manner as treatments.

PAM fluorometry

PAM fluorometry was used *in situ* to assess the effects of macroalgae and their extracts on coral photosynthetic efficiency and bleaching, and the effects of corals on algae (measured as effective quantum yield). PAM fluorometry provides an additional measure of bleaching compared to visual assessments alone, which can be subjective (Fitt et al. 2001, Smith et al. 2006, Pawlik et al. 2007, Rasher and Hay 2010). Values for healthy corals typically range from 0.5 to 0.7 (Fitt et al. 2001); values of ~0.0 to 0.25 indicate severe bleaching and mortality (Smith et al. 2006, Pawlik et al. 2007, Rasher and Hay 2010).

Ideally, researchers utilize dark-adapted corals to minimize variance in yield values associated with nonphotochemical processes, such as UV irradiance and water temperature (Fitt et al. 2001). However, we conducted our study *in situ* on light-adapted corals because of logistical constraints. So that readings for a treatment would not be confounded by variance in environmental parameters, we took all readings between 0900-1400 h and interspersed readings of all treatments through time. Low variance among replicates that were interspersed through time (Figure 2.1), significant correlations between fluorometric and visual assessments of bleaching for all corals (Figure 2.2), and our demonstration of large and significant differences among treatments indicate that treatment effects overwhelmed whatever uncontrolled variance occurred from not using dark-adapted corals.

Algal mimic study

We used inert algal mimics to assess effects of algal abrasion and shading in the absence of chemical effects. To mimic *Padina boryana* (a broad, foliose alga with high potential to shade) we constructed opaque foliose mimics from black plastic sheeting and grouped them with cable-tie "holdfasts." A filamentous mimic of *Chlorodesmis fastigiata* (the most allelopathic alga) was created by cutting 60 loops of Dacron line (White River Fly Shop) into filaments and grouping them with cable-tie "holdfasts". Mimics of *Sargassum polycystum* and *Turbinaria conoides* were not created, because these algae had no effect during our 20 d field experiments. Algal mimics (n = 10 treatment^{-1}) were inserted into segments of three-stranded rope and attached to fragments of *A. millepora* (a coral species that showed high sensitivity to direct macroalgal contact; Figure 2.1B) on racks at Votua Reef, Fiji. We also deployed control corals (n = 10) with rope segments lacking an algal mimic. We assessed the effects of algal mimics vs. controls on coral bleaching and photosynthesis after 16 d. We chose a 16 d duration because live macroalgae had shown strong effects after only 2-10 d (Figure A.1).

Allelochemical bioassays

We exhaustively extracted whole tissues (20 mL displacement volume) of each alga with methanol, filtered each extract, and dried each extract by rotary evaporation. We then partitioned each extract between water and ethyl acetate three times, yielding an ethyl acetate-soluble fraction that was dried by rotary evaporation and stored at -5^{o}C for 2-3 d until assayed.

For bioassays, we re-suspended the ethyl acetate-soluble extract of each alga in methanol and added them at natural volumetric concentration to Phytagel (Sigma-Aldrich) squares (1 cm^2) that were formed on window screen (Thacker et al. 1998).

Controls contained methanol but no extract. In the field, we applied each gel square (n = 10 treatment^{-1}) around a coral branch (fragmented as described above) and secured the square with a cable tie. After 24 h, we removed each gel and took a PAM fluorometry reading beneath its center.

To assess whether allelochemicals were on algal surfaces at concentrations that produced effects we observed in bioassays of whole-algal tissue extracts, we also extracted hydrophobic compounds from only the surfaces of three strongly allelopathic macroalgae and one less allelopathic alga (*i.e.*, it affected some corals but not others) using the "hexane dip" method (Nylund et al. 2007). Each alga (20 mL displacement volume) was spun in a salad spinner to remove excess water and extracted with hexanes for 30 s while vortexing (Nylund et al. 2007). We then dried each extract by rotary evaporation, re-suspended it in hexanes, and added it at natural volumetric concentration to Phytagel squares. Controls contained hexanes but lacked algal extract. Treatment and control gels (n = 10 extract^{-1} coral species^{-1}, except for *Montipora*, n = 5) were deployed and assayed as described above.

Surface extraction verification

To assess whether our methods of surface extraction lysed cells and may have extracted internal chemical constituents, we quantified cell lysis on the surfaces of two allelopathic algae using epifluorescence microscopy (Nylund et al. 2007). Individuals (n = 5) of *C. fastigiata* and *Galaxaura filamentosa* were vortexed in hexanes or seawater for 30 s and immediately preserved in 10% formalin in seawater. In the laboratory, a small piece (5-10 mm in length) of each *G. filamentosa* treatment or control was assessed quantitatively for epithallial cell lysis at a magnification of 200x (Olympus BX41

microscope with a BP420-480 excitation filter, DM500 dichromatic mirror, and BA520IF barrier filter). The number of lysed vs. intact cells on the first filament to appear in view was assessed for 10 randomly selected fields (0.25 mm^2) per individual. Percent lysis was averaged across the ten fields of view for each individual and the mean values for each of the five separate individual algae were used as independent replicates in our analyses. Because *C. fastigiata* is siphonous and lacks discrete cell walls, we cut a small cluster of filaments (15 mm in length) from each treatment and control individual and qualitatively examined the first apical section to appear in view for 10 randomly selected fields (0.25 mm^2) for signs of cell membrane rupture and differences in general cell condition. The percentage of damaged apices (out of 10) was calculated for each individual.

Allelochemical isolation

Methanol extracts of *C. fastigiata* and *G. filamentosa* were separated by reversed-phase Diaion HP20ss (Supelco Analytical) chromatography into four fractions using aqueous methanol and acetone. Based on field bioassay activity, the least polar fraction from each species was further separated with silica gel chromatography, eluting with hexanes/ethyl acetate and methanol. Active fractions from each species were then separated by two rounds of reversed-phase HPLC (C_{18}, 250 x 10 mm, 5µm; Alltech Altima) using a gradient of 70% methanol [aqueous (aq)] to 100% methanol over 60 min and then isocratic 50% methanol (aq) over 35 min to yield allelopathic compounds **1** and **2** from *G. filamentosa*. A gradient of 95% methanol (aq) to 100% methanol over 46 min followed by isocratic 100% methanol over 35 min yielded allelopathic compounds **3** and **4** from *C. fastigiata*.

Structure determination

Compounds **1-4** were isolated in total quantities of 5-18 μg. ^{1}H nuclear magnetic resonance (NMR) spectral data and high-resolution mass spectral (HRMS) data of allelopathic compounds isolated from *G. filamentosa* did not match any known natural products from that genus. For compound **1**, high-resolution electron spray ionization mass spectroscopy (HR-ESI-MS) [M + Na] *m/z* equaled 237.1114 (calculated for $C_{11}H_{18}O_4Na$, 237.1103); for compound **2**, HR-ESI-MS [M + Na] *m/z* equaled 219.1021 (calculated for $C_{11}H_{16}O_3Na$, 219.0997). However, 1D and 2D NMR spectral data for compounds **1** and **2**, recorded on a Varian 800 MHz NMR spectrometer (Agilent Technologies), were compared with and matched with spectral data from compounds isolated from the brown alga *Undaria pinnatifida* (Kimura and Maki 2002). ^{1}H and ^{13}C NMR spectral data for compounds **1** and **2** are reported in Table A.2, with ^{13}C NMR assignments based upon heteronuclear single-quantum correlation spectroscopy and heteronuclear multiple bond correlation data. Carbon positions are noted in Figure 2.5.

^{1}H NMR spectral and HRMS data for allelopathic compounds **3** and **4** isolated from *C. fastigiata* were compared with and matched with previously reported spectral data from *C. fastigiata* (Wells and Barrow 1979, Paul and Fenical 1985). For compound **3**, HR-ESI-MS [M + Na] *m/z* equaled 413.2657 (calculated for $C_{24}H_{38}O_4Na$, 413.2667); for compound **4**, HR-ESI-MS $[M + H]^+$ *m/z* equaled 417.2425 (calculated for $C_{24}H_{33}O_6$, 417.2277). ^{1}H NMR values for compounds **3** and **4** are reported in Table A.3; carbon positions are noted in Figure 2.5.

Compound quantification: surface vs. whole-algal extracts

We assessed the presence of compounds **1-4** and attempted to assess their relative abundance in surface-only vs. whole-algal crude extracts using liquid chromatography-mass spectroscopy (LC-MS). Surface-only and whole-alga extracts (n = 3, 100 mL equivalent for each) were generated using hexanes (for surface extracts) and 100% methanol (for total extracts) as described above. Before analysis, whole-algal crude extracts were fractionated using Diaion HP20ss chromatography (as above), and fractions eluding with 100% methanol (F3) and 100% acetone (F4) were retained. For *G. filamentosa*, fractions F3 and F4 were each separated further into two fractions by reversed-phase HPLC (C_{18}, 250 x 10 mm, 5µm; Alltech Altima) using a gradient of 70% methanol (aq) to 100% methanol over 35 min. LC-MS was performed on a Waters Separation Module 2695 with a reversed-phase column (C_{18} 150 x 3 mm, 3µm; Luna), coupled to a Waters Photodiode Array 2996 and Waters ZQ2000 electron spray ionization mass spectrometer. Analysis of each extract (surface crude or whole-algal crude fractions, n = 3 each) was performed using a gradient of 50% acetonitrile (aq) to 95% acetonitrile (aq) (with 0.01% acetic acid) over 50 min. Peaks identified as compounds **1-4** [by verification of mass (m + H or m + Na) and retention time, relative to LC-MS analysis of each pure molecule] were integrated to calculate the relative abundance of each molecule in each extract. If integration was problematic because of low compound abundance, only the presence of the compound was verified at the appropriate retention time.

Statistical analysis

Coral response data from our competition and allelochemical bioassays violated parametric assumptions and so were evaluated using Kruskal-Wallis Analysis of

Variance (ANOVA) on ranks. Algal response data from our competition bioassays were analyzed by one factor ANOVA or by Kruskal-Wallis ANOVA on ranks if parametric assumptions were violated. If some replicates lost algae or were missed when scoring our 20 d competition study, we randomly excluded replicates from other treatments and controls (~1 to 2) to equalize sample sizes and allow use of more powerful post-hoc tests that require balanced sample sizes. We analyzed the algal mimic assay results with one factor ANOVA. Differences among subgroups were analyzed for all ANOVAs using Student-Newman-Kuels multiple comparisons tests. Relationships between photosynthetic efficiency and coral bleaching were analyzed via Pearson's correlation coefficients. Binomial coral mortality data were analyzed using the Fisher's exact test. Epifluorescent microscopy data we analyzed using the Mann-Whitney U rank sum test.

Results

Algal-coral competition

When placed in contact with eight common macroalgae for 20 d, all three corals experienced bleaching and suppression of photosynthetic efficiency due to contact with some macroalgae (Figure 2.1A-F). Visual bleaching and photosynthetic efficiency were correlated for all three corals ($r = -0.80$ to -0.96, $p < 0.001$ for all comparisons; Figure 2.2); thus, PAM fluorometry measurements were indicative of visual bleaching but are less subjective (Fitt et al. 2001, Smith et al. 2006, Pawlik et al. 2007, Rasher and Hay 2010). The most resistant coral was *Montipora digitata*; for this coral, *Dictyota bartayresiana*, *Galaxaura filamentosa*, and *Chlorodesmis fastigiata* caused significant bleaching and suppression of photosynthetic efficiency, whereas *Liagora* sp. and *Padina*

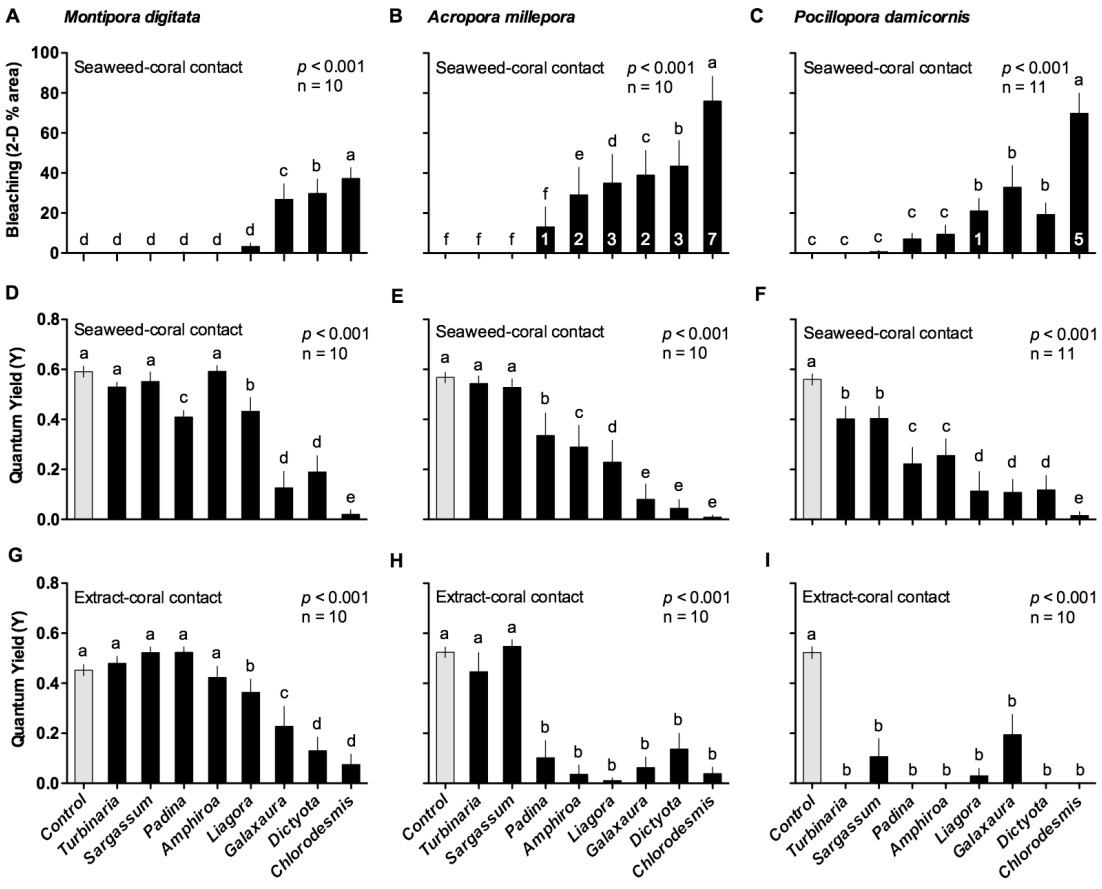

Figure 2.1: Effects of macroalgae and algal extracts on corals. (A-C) Coral bleaching (2-D % area; mean ± SE) and (D-I) effective quantum yield (Y; mean ± SE) of three species of corals when in contact with macroalgae for 20 d (A-F), or in contact with gel squares containing hydrophobic extracts from the same algae for 24 h (G-I), relative to controls (n = 10-11). Analyzed by Kruskal-Wallis Analysis of Variance on ranks. Letters indicate significant groupings by post-hoc Student-Newman-Kuels tests. Numbers inset within bars indicate number of replicates experiencing 100% mortality.

boryana caused no significant bleaching and a mild suppression of photosynthetic efficiency (Figure 2.1A & D). In contrast, contact with *Amphiroa crassa*, *Sargassum polycystum*, or *Turbinaria conoides* had no significant effect on *M. digitata*. Regardless of algal species, significant bleaching occurred only in areas of direct contact and never on the far sides of *M. digitata* 5-10 mm away from algal contact (Kruskal-Wallis ANOVA: $p \geq 0.18$).

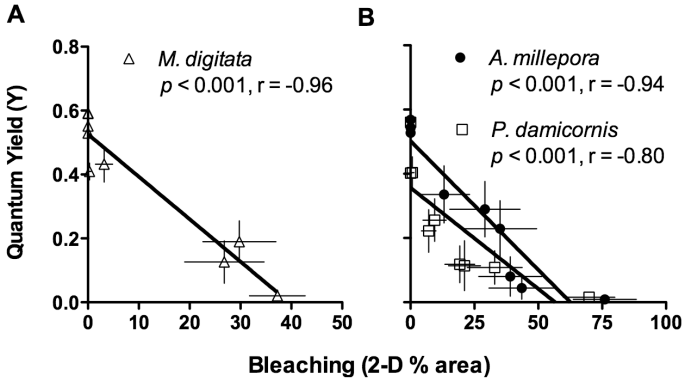

Figure 2.2: Relationship between coral bleaching and photosynthetic efficiency. Linear correlation between coral tissue bleached (2-D % area; mean ± SE) and effective quantum yield (Y; mean ± SE) for (A) *Montipora digitata* (Δ), and (B) *Acropora millepora* (●) and *Pocillopora damicornis* (□) when in direct contact with macroalgae or controls for 20 d. Each point represents a mean (n = 10-11) for that coral species against 1 of the 8 macroalgae or the rope control. Analyzed by Pearson's correlation coefficients. Note scale difference between x-axes.

The corals *Acropora millepora* and *Pocillopora damicornis* were more susceptible to algal damage. For *A. millepora*, all macroalgae but *S. polycystum* and *T. conoides* bleached corals or suppressed photosynthetic efficiency (Figure 2.1B & E); for *P. damicornis*, four of the eight macroalgae caused significant bleaching but all eight

suppressed photosynthetic efficiency (Figure 2.1C & F). Several macroalgae caused mortality of some *A. millepora* and *P. damicornis* replicates (Figure 2.1B & C). However only *C. fastigiata* caused significant whole-replicate mortality of *A. millepora* (Fisher's exact test: $p = 0.003$) and *P. damicornis* ($p = 0.035$). Only *C. fastigiata* caused bleaching on the far sides of *A. millepora* (Kruskal-Wallis ANOVA: $p = 0.021$) or *P. damicornis* ($p = 0.042$), 5-10 mm away from contact. In contrast, corals did not damage macroalgae. Macroalgae in contact with corals experienced no significant bleaching or suppression of photosynthesis relative to controls lacking coral contact (Figure A.2).

Elucidation of competitive mechanisms

Algal effects on corals were largely localized to areas of direct contact. These effects could occur from shading, abrasion, or transfer of hydrophobic allelochemicals upon contact. When inert plastic models mimicking bladed algae such as *P. boryana* and filamentous species such as *C. fastigiata* were put in contact with *A. millepora* (the most sensitive coral, n = 10) for 16 d in the field, mimics produced neither coral bleaching (*Padina* and *Chlorodesmis* mimics: 0 ± 0% bleached; mean ± SE), nor suppression of photosynthetic efficiency (*Padina* mimic: quantum yield (Y) = 0.639 ± 0.013; *Chlorodesmis* mimic: Y = 0.648 ± 0.017) relative to controls lacking a mimic (Y = 0.630 ± 0.014; ANOVA, bleaching: $F_{2, 27} = 1.000$, $p > 0.999$; ANOVA, quantum yield: $F_{2, 27} = 0.295$, $p = 0.747$). In contrast, the alga *C. fastigiata* significantly suppressed *A. millepora* photosynthesis after only 2 d, and five of the eight macroalgae suppressed the coral after only 10 d (Figure A.1), suggesting that allelopathy rather than shading or abrasion damaged corals in our field assays.

Consistent with an allelopathic mechanism, hydrophobic algal extracts placed in contact with corals at natural volumetric concentration for 24 h produced effects (Figure 2.1G-I) that paralleled or exceeded effects of macroalgal contact after 20 d (Figure 2.1D-F). Control gels lacking algal extract had no detectable effect on corals (Figure 2.1G-I). Macroalgae that did not bleach corals in the field also had no effect in assays using their extracts. *Padina boryana* was unusual in that it suppressed photosynthetic efficiency of *M. digitata* during 20 d assays using algal thalli, but its extract was not allelopathic over 24 h. Its allelopathic compounds may be unstable or take longer than 24 h to affect this coral, or it may stress corals mildly through nonchemical mechanisms.

When deployed at natural concentration for 24 h, hydrophobic extracts from only algal surfaces (Figure 2.3) produced effects that mirrored effects of algal thalli and of hydrophobic extracts from whole-algal tissues (Figure 2.1), indicating that hydrophobic compounds occur on algal surfaces at concentrations sufficient to cause coral bleaching and mortality. This assertion could be in error if surface extraction caused cell lysis and extracted internal compounds, but microscopic evaluations of surface extracted *C. fastigiata* and *G. filamentosa* (the most allelopathic algae) indicated that cell lysis did not occur during the extraction process (Table A.1).

Isolation of allelochemicals

Using bioassay-guided chromatographic separations of *G. filamentosa* and *C. fastigiata* crude extracts (15-24 g of extract), we purified and identified four allelopathic compounds - the degraded sesquiterpenes 6-hydroxy-isololiolide and isololiolide from *G. filamentosa* (Figure 2.4A-D; Figure 2.5, **1** and **2**), and the acetylated diterpenes (*E*)-2-((3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl)but-2-ene-1,4-diyl diacetate and (1*E*,3*E*)-

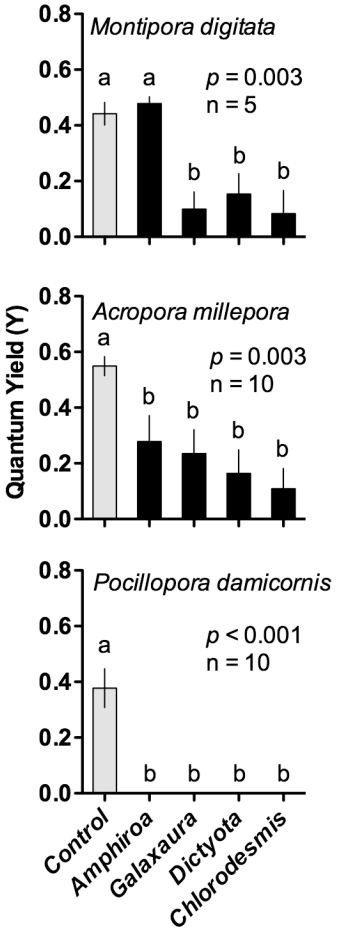

Figure 2.3: Effects of surface-bound algal extracts on corals. Effective quantum yield (Y; mean ± SE) of three coral species when in direct contact for 24 h with gel squares containing hydrophobic extracts from the surfaces of macroalgae, relative to controls (n = 5-10). Analysis and symbols as in Figure 2.1.

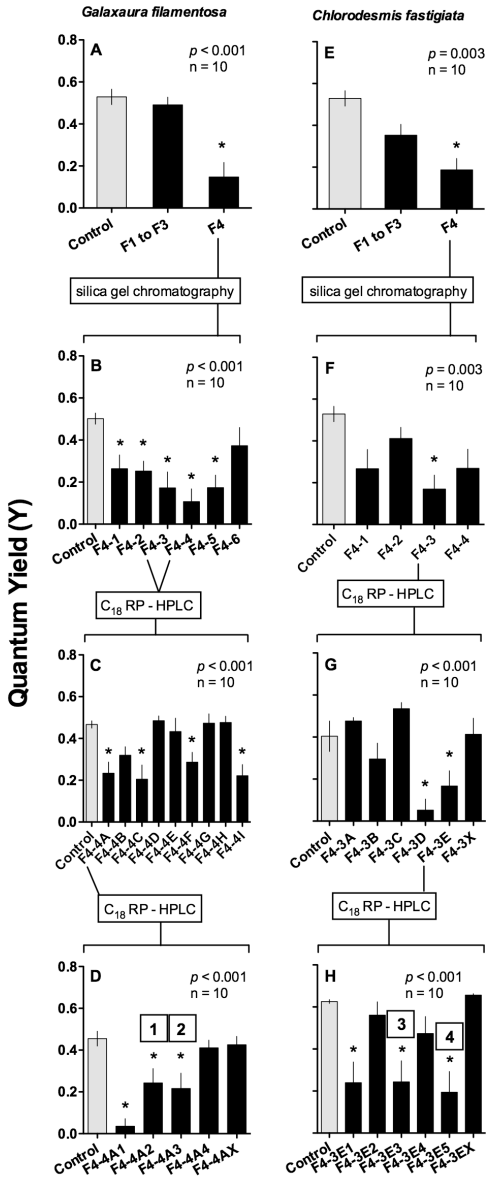

Figure 2.4: Effects of extract fractions on corals. Effective quantum yield (Y; mean ± SE) of the coral *Acropora millepora* when in contact with extract fractions from (A-D) *Galaxaura filamentosa* or (E-H) *Chlorodesmis fastigiata* for 24 h (n = 10 fraction^{-1}), relative to controls. Methanol-soluble crude extracts of both algal species were fractionated by HP20ss reversed-phase chromatography prior to initial bioassay (A & E). Brackets indicate path and method of subsequent extract fractionation based on bioactivity. Stars indicate significant ($p < 0.05$) differences between fractions and controls by post-hoc Student-Newman-Kuels tests. Bioassays lead to the isolation of allelopathic compounds **1** and **2** from *G. filamentosa*, and **3** and **4** from *C. fastigiata*.

2-((3*E*,7*E*)-12-formyl-4,8-dimethyl-10-oxotrideca-3,7,12-trienyl)buta-1,3-diene-1,4-diyl diacetate from *C. fastigiata* (Figure 2.4E-H; Figure 2.5, **3** and **4**). Compounds **1**-**4** were isolated and bioactive at 0.032-0.12 µg/g of algal dry mass, indicating strong potency. Despite these minute concentrations, compounds **1**-**4** were detected in the surface extracts of their associated algae (Figures A.3 and A.4). Compound **3** occurred in *C. fastigiata*

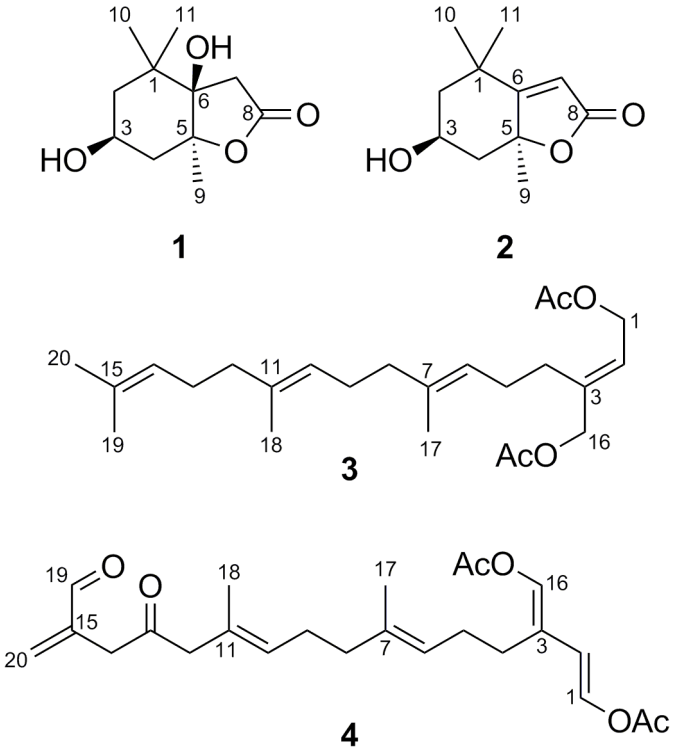

Figure 2.5: Allelopathic compounds isolated from *Galaxaura filamentosa* (**1** and **2**) and *Chlorodesmis fastigiata* (**3** and **4**). Assigned carbon positions are noted for each molecule, and corresponding NMR spectroscopic data are available in Tables A.2 and A.3.

surface extracts (n = 3) at 0.43-7% of its abundance in whole-algal extracts (n = 3); compounds **1**, **2** and **4** were detected in low concentrations in both surface (Figures A.3

and A.4) and whole-algal crude extracts (n = 3) of *G. filamentosa* and *C. fastigiata* (respectively) but internal vs. external concentrations could not be compared rigorously because of their minor abundance relative to other molecules. Additional allelopathic compounds were present in both macroalgae (Figure 2.4). We attempted to identify these molecules, but failed to do so because of low yield or degradation following purification.

Discussion

Direct contact between eight common macroalgae and three genera of common corals caused visible coral bleaching in 50%, suppression of coral photosynthetic efficiency in 79%, and complete death of some coral replicates in 33% of the 24 algal-coral interactions examined (Figure 2.1). In contrast, none of the macroalgae we tested bleached or experienced suppressed photosynthesis due to coral contact (Figure A.2). Field patterns of coral damage were reproduced, or exceeded, in 96% of our bioassays using hydrophobic whole-tissue extracts and hydrophobic surface extracts from these macroalgae; we detected no negative effects of shading or abrasion using inert algal mimics. Additionally, larger and more abrasive macroalgae such as *Turbinaria conoides* and *Sargassum polycystum* that should have produced the greatest abrasion and shading had no detectable effect (vs. *Montipora digitata* and *Acropora millepora*) or minimal effect (vs. *Pocillopora damicornis*) on corals (Figure 2.1). In contrast, some soft, nonabrasive algae with allelopathic extracts (*e.g.*, *Dictyota bartayresiana* and *Chlorodesmis fastigiata*) rapidly bleached and sometimes killed corals within only 2-10 d (Figure A.1). Our findings document allelopathic rather than physical mechanisms mediating these interactions.

A previous study discovered similar patterns for these macroalgae and the coral *Porites cylindrica* in Fiji (Rasher and Hay 2010), but no allelopathic compounds were identified, and it was not possible to assess whether that single coral species was typical or unusual relative to other corals. The present more inclusive study demonstrates that chemical mediation of algal-coral competition is common, and although the magnitude of algal effects vary among corals, some macroalgae (*Dictyota bartayresiana, Galaxaura filamentosa, Chlorodesmis fastigiata, Lobophora variegata, Halimeda opuntia*) are chemically damaging to most corals (Figure 2.1; Rasher and Hay 2010), and others (*T. conoides* and *S. polycystum*) are more chemically benign. These results suggest that macroalgal allelopathy against corals may be common on degraded reefs dominated by certain macroalgae and point to a potentially widespread mechanism to help explain why corals fail to recover on many reefs with abundant macroalgae (Mumby and Steneck 2008, Hughes et al. 2010).

For two of the most allelopathic macroalgae, we isolated multiple allelopathic compounds but were able to identify only two from each species (Figures 2.4 and 2.5). These isoprenoid natural products were known previously from macroalgae (Wells and Barrow 1979, Kuniyoshi 1985, Paul and Fenical 1985, Kimura and Maki 2002, Handley and Blackman 2005); here we elucidate their ecological role. Isolated allelopathic isoprenoids were effective at yields of only 0.032-0.12 μg/g of algal dry mass, suggesting that only minute quantities on algal surfaces can damage corals. The hydrophobicity of these allelochemicals likely makes them efficient surface mediated toxins because their water solubility is very low, allowing these compounds to be retained at algal-coral interfaces. Allelopathic interactions documented among other reef species also occur as

the result of transfer of compounds by contact rather than dissolution through the water (de Nys et al. 1991, Thacker et al. 1998, Kubanek et al. 2002, Pawlik et al. 2007, Rasher and Hay 2010), indicating that in general allelopathic compounds may be hydrophobic rather than hydrophilic in benthic marine systems.

Coral species varied in susceptibility to macroalgal allelopathy (Figure 2.1; Rasher and Hay 2010). *Acropora millepora* and *P. damicornis* were more sensitive to allelopathic damage than *M. digitata* (Figure 2.1) or *P. cylindrica* (Rasher and Hay 2010). Moreover, mortality occurred only for some replicates of *A. millepora* (23%) and *P. damicornis* (8%) and never for *M. digitata* or *P. cylindrica* (Figure 2.1; Rasher and Hay 2010). Despite some inconsistencies associated with taxonomic relatedness (see responses of *A. millepora* vs. *M. digitata* to algal contact, both in the family Acroporidae), these differences in coral sensitivity to algal allelopathy parallel the differing tolerances of the genera *Acropora* and *Porites* to climate-induced bleaching and mirror the high extinction risk of *Acropora* and stability of *Porites* at a global scale (Carpenter et al. 2008). Therefore, differential effects of macroalgae on corals may reinforce trajectories of coral decline initially produced by large-scale disturbance.

Macroalgae like *S. polycystum*, *T. conoides*, and *Padina boryana* that commonly bloom following herbivore removal (Lewis 1986, Hughes 1994, Hughes et al. 2007) did not damage corals in our assays. Because we used only one thallus per coral, it is possible that higher densities of these macroalgae or longer contact durations could damage corals (Hughes et al. 2007). In contrast, macroalgae such as *D. bartayresiana* (Figure 2.1) or *L. variegata* (Rasher and Hay 2010) that also commonly bloom following herbivore exclusion (Hughes 1994, Burkepile and Hay 2008, Sotka and Hay 2009) had

large allelopathic effects on corals, and could thus reduce coral resilience (Mumby and Steneck 2008, Hughes et al. 2010). However, not all species in these genera are allelopathic (Box and Mumby 2007), making generalizations based on relatedness or functional group alone problematic. Interestingly, other strongly allelopathic macroalgae such as *C. fastigiata*, *G. filamentosa*, *Liagora* sp. (Figure 2.1) or *Ochtodes secundaramea* (Rasher and Hay 2010) rarely proliferate following reef disturbance. Differential susceptibility to disturbance (herbivory, hydrodynamics, and other factors) or differential competitive ability may explain in part why some chemically damaging macroalgae rarely bloom on declining reefs (Burkepile and Hay 2008, Hoey and Bellwood 2009, Rasher and Hay 2010).

Recent laboratory studies demonstrated that macroalgae near but not in contact with corals triggered coral mortality, and suggested that algae release water-soluble compounds that kill corals indirectly by stimulation of harmful coral-associated microbes (Smith et al. 2006). Field studies indicate that benthic algae release hydrophilic molecules, such as dissolved organic carbon (DOC), capable of fueling these interactions (Haas et al. 2010, Hauri et al. 2010). However, in our field assays where advection would disperse and dilute such compounds rapidly, we did not detect macroalgal effects beyond sites of direct contact for either *M. digitata* (Figure 2.1) or *P. cylindrica* (Rasher and Hay 2010) as would be expected for interactions involving hydrophilic molecules. We detected algal effects beyond areas of direct contact for the more chemically sensitive corals (*A. millepora* and *P. damicornis*) when contacted by *C. fastigiata*, but the allelopathic effects of this alga and the alga *G. filamentosa* were traced from methanol-soluble crude extracts (containing both hydrophobic and hydrophilic molecules) to potent

hydrophobic compounds that would be minimally dispersed by water (Figure 2.4). Water-soluble compounds within the polar fractions (F1 to F3) of *C. fastigiata* and *G. filamentosa* crude extracts were not active in bioassays (Figure 2.4A & E). Thus, in our field studies most algal damage to corals appeared to be caused by hydrophobic molecules transferred by direct contact, suggesting that water-soluble compounds such as DOC need not be involved in these contact allelochemical interactions [although water-soluble compounds might be involved under some conditions (Hauri et al. 2010)]. Whether the hydrophobic compounds and extracts we detected poison corals directly or indirectly by altering microbial communities (Nugues et al. 2004, Smith et al. 2006) was not assessed.

Algal-coral interactions may have been rare on pristine or "pre-human" reefs where herbivores limited macroalgae to spatial refuges (Jackson et al. 2001, Pandolfi et al. 2003); thus, factors historically selecting for macroalgal allelopathy against corals remain unclear. Given that macroalgae share a long evolutionary history with microbial pathogens (Goecke et al. 2010), that the isoprenoid compounds we identified are found in several green (Wells and Barrow 1979, Paul and Fenical 1985, Handley and Blackman 2005), red (Figures 2.4 and 2.5), and brown macroalgae (Kuniyoshi 1985, Kimura and Maki 2002), and that some of these compounds suppress marine microbes (Paul and Fenical 1985), it is plausible that these compounds evolved independently in multiple algal lineages as defenses against microbes. These natural products may damage corals fortuitously and thus provide an advantage to macroalgae on present-day reefs.

Recent analyses suggest that marine protected areas may promote local processes, such as herbivory and coral recruitment (Mumby et al. 2006, Mumby et al. 2007) that

limit macroalgal proliferation and promote coral recovery, thereby bolstering coral resilience to large-scale disturbance (Hughes et al. 2007, Carilli et al. 2009, Hughes et al. 2010, Mumby and Harborne 2010, Selig and Bruno 2010). However, because several allelopathic macroalgae (*e.g.*, *Dictyota*, *Chlorodesmis*, *Galaxaura, Lobophora, Halimeda, Ochtodes*) also contain toxins that deter some herbivores (Schupp and Paul 1994), developing effective marine reserves may require protecting a diverse herbivore guild that includes species that consume chemically defended macroalgae (Schupp and Paul 1994, Burkepile and Hay 2008, Rasher and Hay 2010). Establishing no-take reserves or fishing bans that protect herbivores capable of consuming chemically rich macroalgae may minimize allelopathic effects of algae on corals.

We show that numerous common macroalgae damage a variety of corals using surface-associated allelochemicals transferred by algal-coral contact; these interactions will be especially detrimental to the recovery of small remnant coral colonies or to the survivorship of small juvenile corals encountering a high ratio of algal contact per unit area. Such interactions also may contribute to the algal suppression of coral fecundity and recruitment documented in previous investigations (Birrell et al. 2008, Foster et al. 2008, Diaz-Pulido et al. 2010) and may become increasingly damaging to corals as oceans acidify (Diaz-Pulido et al. 2011). If so, chemically mediated algal-coral competition may play a critical and increasing role in both the degradation of coral reefs and the formation of negative feedbacks limiting reef recovery (Birrell et al. 2008, Mumby and Steneck 2008). Understanding which macroalgae most harm corals and what processes limit these macroalgae may allow more proactive management that increases coral resilience

to the many stresses impacting tropical reefs (Mumby and Steneck 2008, Hughes et al. 2010).

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CHAPTER 3

EFFECTS OF HERBIVORY, NUTRIENTS, AND REEF PROTECTION ON ALGAL PROLIFERATION AND CORAL GROWTH ON A TROPICAL REEF

Abstract

Maintaining coral reef resilience against increasing anthropogenic disturbance is critical for effective reef management. Resilience is partially determined by how processes such as herbivory and nutrient supply affect coral recovery vs. macroalgal proliferation following disturbances. However, the relative effects of herbivory vs. nutrient enrichment on algal proliferation remain debated. Here we manipulated herbivory and nutrients on a coral-dominated reef protected from fishing, and on an adjacent macroalgal-dominated reef subject to fishing and riverine discharge, over 152 days. On both reefs, herbivore exclusion increased total and upright macroalgal cover by 9-46 times, upright macroalgal biomass by 23-84 times, and cyanobacteria cover by 0-27 times, but decreased cover of encrusting coralline algae by 46-100% and short turf algae by 14-39%. In contrast, nutrient enrichment had no effect on algal proliferation, but suppressed cover of total macroalgae (by 33-42%) and cyanobacteria (by 71% on the protected reef) when herbivores were excluded. Herbivore exclusion but not nutrient enrichment also increased sediment accumulation, suggesting a strong link between herbivory, macroalgal growth, and sediment retention. Growth rates of the corals *Porites cylindrica* and *Acropora millepora* were 30-35% greater on the protected vs. fished reef, but nutrient and herbivore manipulations within a site did not affect coral growth. Cumulatively, these data suggest that herbivory rather than eutrophication plays the

dominant role in mediating macroalgal proliferation, that macroalgae trap sediments that may further suppress herbivory and enhance macroalgal dominance, and that corals are relatively resistant to damage from some macroalgae but are significantly impacted by ambient reef condition.

Introduction

Corals, and the reefs they build, are in rapid global decline due to numerous anthropogenic stresses (Bellwood et al. 2004, Knowlton and Jackson 2008, Hughes et al. 2010). Interactions between climate-induced coral bleaching (Hoegh-Guldberg et al. 2007, Baker et al. 2008), coral disease (Bruno et al. 2003, Bruno et al. 2007, Harvell et al. 2007), coastal pollution (Bruno et al. 2003) and the cascading effects of overfishing (Jackson et al. 2001, Bellwood et al. 2004, Raymundo et al. 2009) have lead to dramatic losses of coral over large spatial scales (Hughes et al. 2003, Bellwood et al. 2004, Hughes et al. 2010). Emerging research suggests that overfishing of reef herbivores at local scales limits the capacity of corals to resist or recover from global-scale disturbance (Hughes et al. 2003, Hughes et al. 2007, Mumby and Steneck 2008, Hughes et al. 2010); the loss of herbivores from already-disturbed reefs has commonly been followed by dramatic coral decline and macroalgal proliferation (*i.e.*, a "phase-shift") (Folke et al. 2004, Hughes et al. 2010). Once established, algal-dominated communities limit coral and herbivore recruitment, reduce intensity of herbivory, and thereby reinforce the persistence of algal-dominated communities (Mumby et al. 2007a, Mumby et al. 2007b, Mumby and Steneck 2008, Hughes et al. 2010, Hoey and Bellwood 2011). However, the relative importance of processes mediating macroalgal proliferation and phase-shifts on

reefs are debated (Lapointe et al. 2004, Burkepile and Hay 2006, Littler et al. 2006a, Littler et al. 2006b, Heck and Valentine 2007, Houk et al. 2010, Smith et al. 2010).

Numerous empirical, theoretical, and meta-analytical studies suggest that the "top-down" process of herbivory plays a critical role in determining the abundance and distribution of macroalgae, and the outcome of coral-algal interactions affecting phase-shifts on reefs (Lewis 1986, Jompa and McCook 2002, Burkepile and Hay 2006, Heck and Valentine 2007, Hughes et al. 2007, Mumby et al. 2007b, Burkepile and Hay 2008, Elmhirst et al. 2009, Rasher and Hay 2010). Manipulations of reef herbivores (Lewis 1986, Hughes et al. 2007, Burkepile and Hay 2008), long-term observations of reef decline (Hughes 1994, Cheal et al. 2010), and monitoring of the consequences of reef protection (Mumby et al. 2007a, Mumby and Harborne 2010) all suggest that herbivores strongly suppress macroalgal colonization and growth, lessen algal damage to corals, and promote coral recruitment and growth. For many of these studies, strong herbivore effects were observed even in the presence of elevated nutrient levels that might stimulate algal growth, indicating that herbivory may buffer against increased macroalgal production associated with nutrient enrichment (Burkepile and Hay 2006, Heck and Valentine 2007). However, a few field studies suggest that the "bottom-up" process of nutrient supply can trigger algal proliferation, even in the presence of herbivory, if threshold nutrient levels are exceeded (Lapointe 1997, Smith et al. 2001, Lapointe et al. 2004, Littler et al. 2006a, Littler et al. 2006b). Other studies demonstrate that nutrient enrichment can impact algal proliferation if herbivory is strongly reduced (Burkepile and Hay 2006, Burkepile and Hay 2009, Smith et al. 2010), and if experiments are conducted over sufficient time-scales for nutrient effects to emerge (Smith et al. 2010). Moreover,

small-scale field manipulations may not match large-scale, long-term survey results (Houk et al. 2010) or long-term manipulative studies (Smith et al. 2010), and some authors suggest that results from studies conducted on reefs already dominated by macroalgae may not be typical of reefs that have yet to undergo phase-shifts (Smith et al. 2010). Thus, although the preponderance of data available to-date indicates a greater role for top-down than for bottom-up forces, the relative influences of these forces on algal proliferation can be context-dependent (Burkepile and Hay 2006, Houk et al. 2010, Smith et al. 2010).

This context-dependent nature of top-down vs. bottom-up control of reef community state has created a debate concerning the relative importance of each process, in part due to the limited number of studies that have interactively assessed herbivory and nutrient enrichment, and due to the limited duration and/or scale of most experiments (Burkepile and Hay 2006, Houk et al. 2010, Smith et al. 2010). Additionally, even fewer studies have monitored the cascading effects of these processes on coral recruitment, growth and/or survival (Burkepile and Hay 2009, Sotka and Hay 2009, Houk et al. 2010, Smith et al. 2010). Moreover, studies have rarely assessed the importance of these processes along gradients of environmental stress, such as on fished reefs dominated by macroalgae vs. protected reefs dominated by corals, or among reefs with varying levels of natural or anthropogenic nutrient input - such studies are needed to better evaluate the context-dependency of bottom-up vs. top-down effects (Houk et al. 2010, Smith et al. 2010). Increased knowledge of the cascading effects of herbivore exploitation vs. reef eutrophication is critical for the prioritization of management efforts that increase reef resistance to phase-shifts and/or facilitate reef recovery.

The goals of this study were to: (1) assess the relative influence of top-down (herbivory) vs. bottom-up (nutrient supply) processes on the development of benthic macroalgal communities, (2) determine how these processes differ on coral- vs. macroalgal-dominated reefs, and (3) monitor the cascading impacts of these resultant algal communities on sediment accumulation and coral growth. To accomplish these goals, we conducted field experiments that assessed the individual and interactive effects of herbivore exclusion and nutrient enrichment on macroalgal proliferation, sediment accumulation, and coral growth on a coral-dominated Fijian reef protected from fishing, and on an adjacent macroalgal-dominated reef subject to local artisan fishing and riverine discharge, over 152 days.

Material and Methods

Study site and experimental design

We assessed the effects of herbivore exclusion, nutrient enrichment, and the interaction of these factors on algal community development, sediment accumulation, and coral growth at two shallow reef flat sites (~0.5 Km apart) along the Coral Coast of Viti Levu, Fiji (18º13.049'S, 177º42.968'E), 20 October 2008 to 20 March 2009 (duration = 152 days). Using a fully factorial design [herbivores/no nutrient enrichment (+H-N), herbivores/nutrient enrichment (+H+N), herbivore exclusion/no nutrient enrichment (-H-N), herbivore exclusion/nutrient enrichment (-H+N)], we deployed spatially blocked sets of treatments onto shallow reef flats (~1 m depth low tide; ~2 m depth high tide) (1) within the boundaries of a no-take marine protected area on a minimally developed shoreline (herein referred to as "MPA") and (2) within the boundaries of an adjacent area subject to impacts associated with local artisan fishing, an immediately adjacent village,

and the nutrient/sediment input from a small river that runs by the village (herein referred to as "non-MPA"). Treatments were spatially blocked to control for small-scale variation in herbivory and ambient nutrient supply. The MPA is characterized by 57% coral cover, 3% upright fleshy macroalgal cover, and high rates of macroalgal removal by fishes; the non-MPA is characterized by 3% coral cover, 47% macroalgal cover, and low macroalgal removal rates (Rasher and Hay 2010). Thus, our experimental design allowed us to assess the localized effects of herbivory and nutrient enrichment under differing levels of fishing, adjacent human settlement, and riverine discharge. Treatments within blocks were separated by 1-3 m, while blocks ($n = 10$ site^{-1}) were separated by 20-25 m.

This design allows independence and interspersion of treatments within each larger site, but potentially confounds MPA vs. non-MPA contrasts with location since there is only one larger site of each type. This limitation should be noted, but is reduced somewhat by the close proximity, similar depth, similar orientation, etc. of the two sites. Additionally, villager statements indicate that 30+ years ago, both sites supported high coral and low algal abundance, suggesting similar biotic communities were historically supported at both sites.

Each experimental unit was constructed from a concrete cinder block (~10 x 20 x 40 cm), cemented flat to the reef substrate. The upper surface of each block (800 cm^2) provided a settlement site for benthic organisms, and allowed for the slow diffusion of nutrients to the upper surface of the block for treatments where fertilizers were sealed into the center spaces within each block (Miller et al. 1999, Burkepile and Hay 2009). To exclude large herbivores, we encircled mesh wire (1 cm^2 grid) around each block to form a tube with a diameter of ~50 cm and closed the ends of the tube with the same wire

mesh. To control for shading and hydrodynamic effects of the mesh, but allow for block access to both small and large herbivores, we enclosed "herbivore" treatment blocks within the same types of mesh tubes but left the ends open. Previous studies using this design found no significant difference in algal communities between blocks with partial cages and cage-free blocks (Miller and Hay 1998, Sotka and Hay 2009). Cages were inspected for damage and brushed clean every 30 d.

Nutrient enrichment

To produce nutrient enrichment treatments, we sealed one side of both internal chambers on a block with cement, placed 100 ± 10 g Osmocote (Scotts) commercial slow-release fertilizer pellets (19:6:12, N:P:K) held inside a mesh pouch (L'eggs stockings) within each block chamber, and plugged each of the opposite sides of the block opening with a section of removable closed-cell foam (Miller et al. 1999). Additional nutrients were added every 30 d as previous studies demonstrated that this frequency of addition maintained enhanced nutrient levels (Miller et al. 1999, Burkepile and Hay 2009). As with previous applications of this method (Miller at al. 1999, Burkepile and Hay 2009, Sotka and Hay 2009), our goal was to deliver a localized supply of nutrients to algal tissues growing directly on the experimental surface. Blocks without nutrient enrichment treatments were sealed in the same way, but no nutrients were placed within chambers of those blocks.

To assess the efficacy of our nutrient enrichment treatment, we measured carbon:nitrogen (C:N) ratios within tissues of *Padina boryana* (the most abundant macrophyte) growing on enriched vs. non-enriched blocks excluded from herbivores, at the end of the 152 d study. These same *Padina* tissues were also sampled for elemental

and isotopic (^{15}N, ^{13}C) composition to assess the degree of nutrient limitation between sites, as well as the relative contribution of marine- vs. terrestrially- derived nutrients incorporated into macroalgal tissues from ambient waters.

Algal community development

At the end of the 152 d experiment, we quantified cover of algae on the upper surface (a 20 x 40 cm rectangle = 800 cm^2) of each experimental block by laying a beaded chain over the block surface and identifying algae under each of 60 randomly pre-marked points. Algae were identified to the lowest taxonomic level possible in the field, but most algae were categorized into morphological or taxonomic groups [upright fleshy macrophytes, algal turfs < 0.05 cm, algal turfs > 0.05 cm, cyanobacteria, crustose coralline algae (herein known as "CCA")] because high-resolution taxonomic identification in the field was problematic. Greater than 95% of all upright fleshy macroalgal biomass was *Padina* spp.; thus, upright macroalgae were pooled for analyses. If more than one species was present under a single point (*e.g.*, CCA overgrown by an upright macrophyte), both species were counted; as such, cover could exceed 100%. We also removed upright macroalgae from the top surface of each block (a 20 x 40 cm rectangle = 800 cm^2), transported them to the lab in sealed plastic bags, removed excess water with a salad spinner (10 revolutions), and obtained total wet mass (g) of upright macroalgae. These macroalgal samples were then frozen for elemental and isotopic analysis (see below). Blocks were visually inspected for coral recruits, but none were noted on the blocks. "Total algal cover" (see Figure 3.1A) was calculated as the sum of upright fleshy macroalgae, cyanobacteria, and tall algal turf (> 0.05 cm) cover. We excluded algal turfs < 0.5 cm and CCA from this grouping, as these groups are (1)

unlikely to impact the size class of corals we deployed on our experimental blocks, (2) are unlikely to suppress coral recruitment (Birrell et al. 2008), and (3) are characteristic of healthy reefs with high rates of herbivory (Steneck 1988, Burkepile and Hay 2006). At the end of the experiment, we also scraped sediments and filamentous algae from each block into a plastic bag, brushed and washed each block (above water), and then quantified cover of CCA in the absence of larger algae and sediments that could have obscured cover of CCA. CCA cover was quantified using 100 points set randomly within a 15 x 30 cm quadrat. However, *in situ* and post-scraping point counts did not differ (Wilcoxon signed rank test: $p = 0.155$, n = 80), so *in situ* counts were used for analyses to maintain consistency in scoring. Data for algal cover and biomass violated parametric assumptions, so the effects of herbivores, nutrients, and site on algal accumulation were analyzed with three factor analysis of variance (ANOVA) on rank-transformed data.

Sediment accumulation

Following the scoring of algal percent cover in the field, sediments, small filamentous algae, and small invertebrate infauna were scraped from blocks into plastic bags and frozen for analyses. In the laboratory, each sample was defrosted, transferred to a sieve (1 mm mesh), and water slowly passed through the sample to break up consolidated sediments. Microfauna and flora retained on the sieve were removed. Each sediment-laden water sample was then suctioned through a pre-ashed and -weighed glass fiber filter (Whatman) to trap all particles. Filters holding sediments were then dried to a constant mass (80°C), and ashed (500°C for 12 h) to obtain dry, ash, and ash-free dry masses for each sediment sample.

Elemental and isotopic composition of macroalgae

Returning our frozen macroalgal samples to the laboratory, we measured the elemental (N and C) content and isotopic composition of lyophilized *Padina boryana* samples by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a Micromass Optima interfaced to a CE Elantech NA2500 elemental analyzer. All nitrogen isotope abundances are reported as $\delta^{15}N$ and $\delta^{13}C$ values relative to atmospheric N_2 and VPDB, respectively. Each analytical run included a size series of elemental (methionine) and isotopic (peptone) standards, which provided a check on the stability of the instrument and allowed us to remove the contribution of any analytical blank from our isotopic measurements (Montoya 2008).

Coral growth

We also assessed the effects of herbivore exclusion and nutrient enrichment on coral growth. To monitor growth, we stained 6-8 cm height branches of the corals *Porites cylindrica* and *Acropora millepora* in a 15 mg/L solution of Alizarin red (Sotka and Hay 2009, Burkepile and Hay 2010) for 12 h (4 h day/8 h night) in large coolers filled with seawater, and then epoxied one fragment of each species into equidistant holes drilled on opposite ends of each block surface ($n = 10\ species^{-1}\ treatment^{-1}\ site^{-1}$). At the end of the field experiment, we removed and bleached corals. To assess growth, corals were imbedded into blocks of paraffin wax, and sectioned 2-3 times vertically on a diamond saw (MK Diamond Products). Growth was determined by calculating the two-dimensional, cross-sectional percent area of new growth, relative to the stain demarking initial size, using ImageJ (v.1.40, National Institutes of Health) photo analysis software. Growth quantified for each sectioned piece was averaged within a coral replicate. Some replicates did not incorporate the stain clearly for accurate scoring, or were missing at the

termination of the experiment; these were excluded from the analyses. Data for *Porites* were not normally distributed and for *Acropora* were heteroscedastic, and so were analyzed with three factor analysis of variance (ANOVA) on rank-transformed data.

Results

Effectiveness of nutrient enrichment

Nitrogen was significantly enriched in tissues of *Padina boryana* growing on nutrient enriched vs. non-enriched blocks protected from herbivores, regardless of site (C:N ratios were 22.21 ± 0.64 and 24.19 ± 0.70, respectively; two factor ANOVA, Site: $F_{1, 26} = 0.981$, $p = 0.331$; Enrichment: $F_{1, 26} = 4.996$, $p = 0.034$; SxE: $F_{1, 26} = 1.189$, $p = 0.285$; n = 6-8 treatment^{-1} site^{-1}). Thus, our nutrient enrichment was successful in that nutrients from the blocks were physiologically available to, and used by, macroalgae on enriched blocks. C:N ratios for non-enriched macroalgae did not differ between algae on blocks in the non-MPA vs. MPA; thus, macroalgal access to, or use of, nutrients did not differ between sites despite riverine input and greater human population density near the non-MPA. The δ^{15}N of *Padina* growing on enriched and non-enriched blocks did not differ as a function of our fertilization treatments (n = 6-8 treatment^{-1} site^{1}; two factor ANOVA, Enrichment: $F_{1, 26} = 0.434$, $p = 0.516$), but there was a large effect of site; *Padina* growing on blocks in the non-MPA had a significantly lower δ^{15}N than *Padina* from the MPA (0.90 ± 0.32‰, n = 14 vs. 2.09 ± 0.14‰, n = 16, respectively; two factor ANOVA, Site: $F_{1, 26} = 11.358$, $p = 0.002$), suggesting the sites differed in sources of nutrients. Although *Padina* δ^{13}C tended to be lower in the non-MPA (-11.44 ± 1.22‰, n = 14) than in the MPA (-10.33 ± 1.79‰, n = 16) and lower on enriched blocks (-11.19 ± 1.86‰, n = 16) than on non-enriched blocks (-10.45 ± 1.28‰, n = 14), these differences

were not statistically significant (two factor ANOVA, Site: $F_{1, 26} = 3.320$, $p = 0.080$; Enrichment: $F_{1, 26} = 1.328$, $p = 0.260$), but the trend for a site effect is suggestive.

Effects of herbivore exclusion and nutrient enrichment on algal community development

Exclusion of large herbivores increased the cover of total macroalgae and upright fleshy macroalgae by 9-46 times, increased cover of cyanobacteria by 0-27 times, and decreased cover of CCA by 46-100% and short (< 0.5 cm) algal turfs by 14-39% (Figure 3.1; Table B.1). In contrast, nutrient enrichment did not significantly increase cover of any algal group [although suggestive for short algal turfs in the absence of herbivores ($p = 0.074$)], and suppressed cyanobacteria cover in the MPA by 71%, but only when large herbivores were excluded (Figure 3.1; Table B.1). In the absence of herbivores, nutrient enrichment also suppressed total macroalgal cover by 33-42% as indicated by a significant herbivore x nutrient interaction term (three factor ANOVA, HxN: $p = 0.011$; Figure 3.1A). However, post-hoc analysis did not rigorously detect this difference ($p = 0.058$), but the nearly significant p value is suggestive. When we assessed wet mass rather than percentage cover of upright fleshy macroalgae per 800 cm^2 (the top of each block), the patterns were similar (Figure 3.2); herbivore exclusion increased upright macroalgal mass 23-84 times ($p < 0.001$), while nutrient addition had no detectable effect ($p = 0.769$).

With the exception of cyanobacteria, the placement of experimental blocks in the MPA vs. the non-MPA had no significant effect on algal community development after

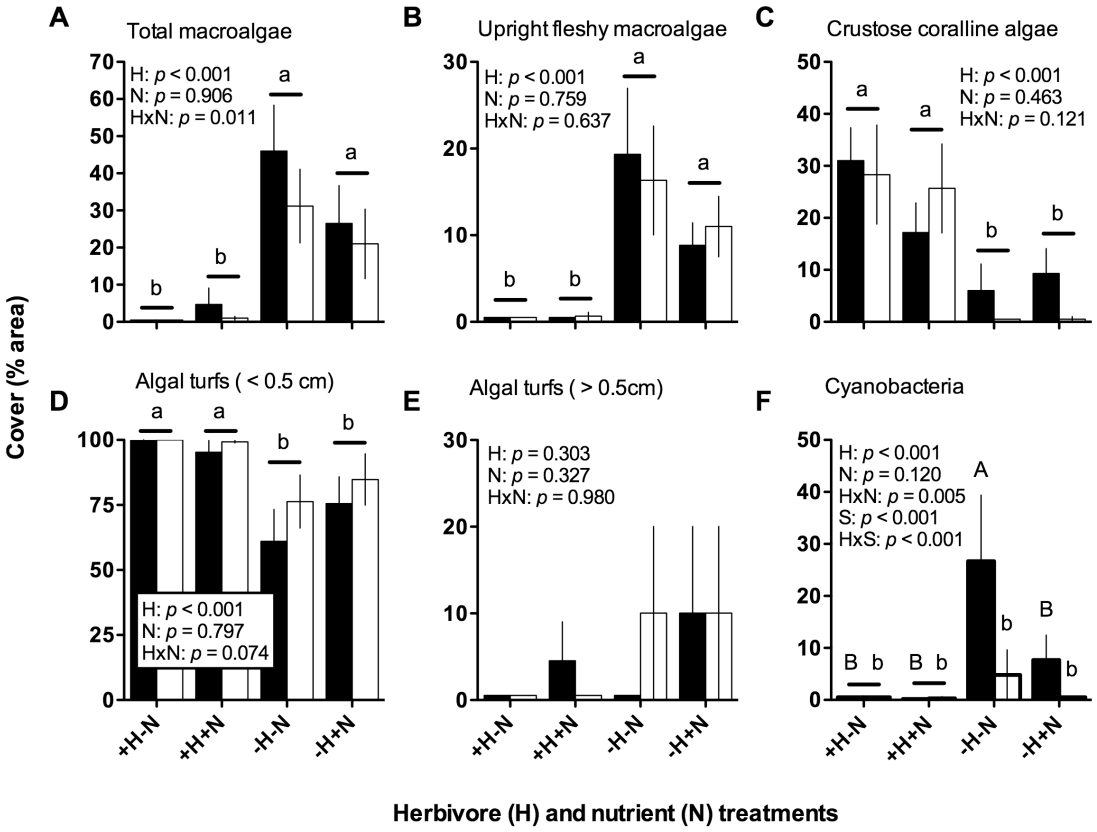

Figure 3.1: Percent cover (mean ± SE) of (A) total macroalgae and (B-F) common algal types on settlement blocks accessible (+H) or inaccessible (-H) to herbivores, both without (-N) and with (+N) nutrient enrichment, when deployed on a reef in a no-take marine protected area (MPA; black bars) or on an adjacent fished reef (non-MPA; white bars) for 152 d (n = 10 treatment^{-1} site^{-1}). *p* values are from three factor analysis of variance (ANOVA) of rank-transformed data. See Table B.1 for complete ANOVA results. Letters indicate significant groupings by Tukey HSD tests. Horizontal bars indicate non-significant differences between sites (S), within a treatment. For (F), upper and lower case letters distinguish contrasts within the MPA and within the non-MPA, respectively. Note scale differences on y-axes.

152 d (Figure 3.1; Table B.1). Cyanobacteria were unusual in that exclusion of herbivores increased cyanobacteria cover for blocks within the MPA, but nutrient addition suppressed this effect to levels similar to treatments including herbivores. In the non-MPA, herbivore exclusion and nutrient enrichment had no effect on cyanobacteria growth (Figure 3.1F).

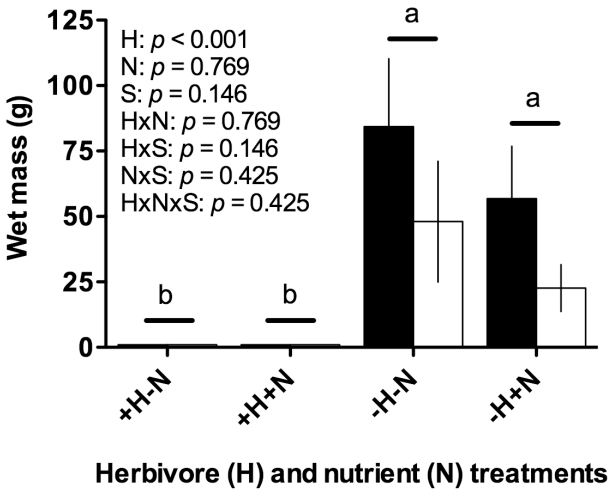

Figure 3.2: Wet mass (grams; mean ± SE) of larger upright fleshy macroalgae on settlement blocks accessible (+H) or inaccessible (-H) to herbivores, both without (-N) and with (+N) nutrient enrichment, when deployed on a protected reef (MPA; black bars) or on an adjacent fished reef (non-MPA; white bars) for 152 d (n = 10 treatment^{-1} site^{-1}). *p* values are from three factor analysis of variance (ANOVA) on rank-transformed data. Letters indicate significant groupings from a Tukey HSD test. Horizontal bars indicate non-significant differences between sites (S), within a treatment.

Effects of herbivore exclusion and nutrient enrichment on sediment accumulation

Excluding large herbivores significantly increased sediment accumulation on experimental blocks; dry mass of inorganic sediments was 66-89% higher and ash-free

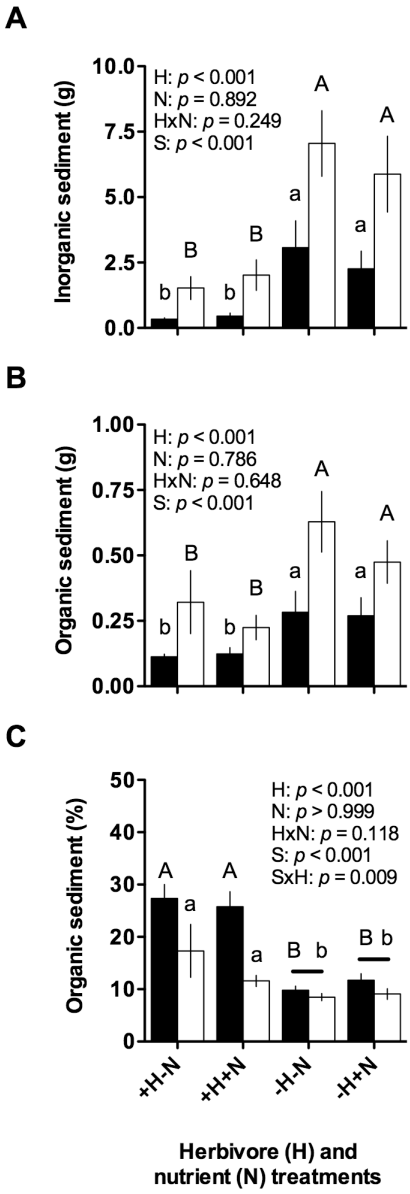

Figure 3.3: (A) Inorganic and (B) organic sediments (grams; mean ± SE), or (C) percentage (mean ± SE) of total sediments that are organic on settlement blocks accessible (+H) or inaccessible (-H) to herbivores, both without (-N) and with (+N) nutrient enrichment, when deployed on a protected reef (MPA; black bars) or on an adjacent fished reef (non-MPA; white bars) for 152 d (n = 10 treatment^{-1} site^{-1}). *p* values are from three factor analysis of variance (ANOVA) of rank-transformed data. See Table B.2 for complete ANOVA results. Letters indicate significant groupings by Tukey HSD tests. Horizontal bars indicate non-significant differences between sites (S), within a treatment. Upper and lower case letters distinguish within-site contrasts among treatments. Note scale differences on y-axes.

dry mass of organic sediments was 49-60% higher on herbivore exclusion blocks than blocks subject to herbivory (Figure 3.3; Table B.2). Nutrient enrichment had no effect on sediment accumulation, but blocks of all treatments accumulated significantly more sediments when deployed within the non-MPA vs. the MPA (Figure 3.3A & B).

Organic contributions to total sediment loads were 22-64% greater on blocks subject to herbivory vs. blocks excluded from herbivores; nutrient enrichment had no effect on the proportion of organic sediments accumulated. Moreover, organic contributions to sediments were significantly greater within the MPA vs. non-MPA, but only for blocks accessible to herbivores (Figure 3.3C; Table B.2).

Effects of herbivore exclusion and nutrient enrichment on coral growth

Neither the exclusion of large herbivores or addition of nutrients, nor their interaction affected the growth of the mounding coral *Porites cylindrica* over the 152 d experimental period. However, *P. cylindrica* growth averaged a significant 30% greater in the MPA than in the non-MPA (Figure 3.4A; Table B.3). Although the faster growing, tabular coral *Acropora millepora* grew 27-41% more on blocks subject to grazing by large herbivores (with or without nutrient enrichment), this effect was suggestive but not statistically significant ($p = 0.075$) (Figure 3.4B; Table B.3). Our power to detect among-treatment differences for *Acropora* was compromised due to unexplained deaths of 9 of 40 outplants in the MPA and 2 of 40 in the non-MPA within the first month of our experiment; after this initial death, survivorship of *Acropora* was high (> 98%). Like *Porites, A. millepora* growth averaged a significant 41% greater when deployed in the MPA vs. the non-MPA (Figure 3.4B; Table B.3).

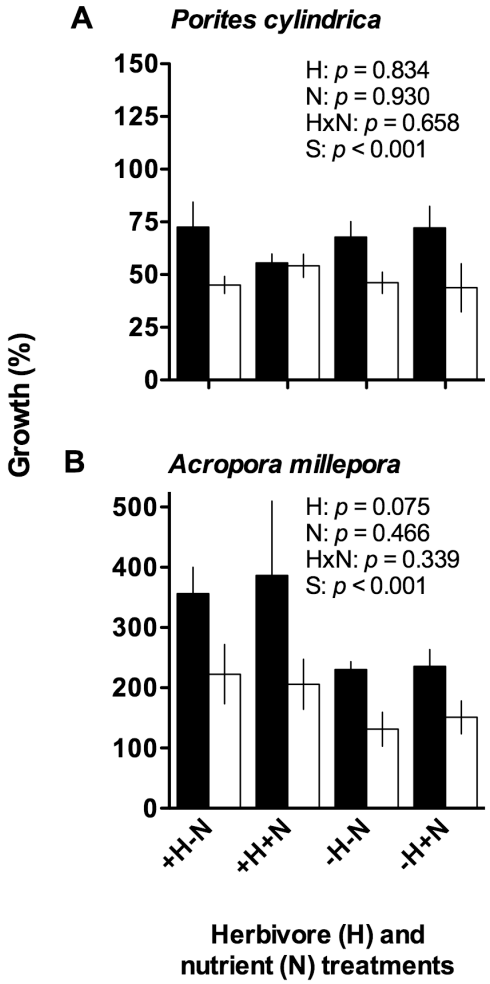

Figure 3.4: Percent growth (2-D, cross-sectional area; mean ± SE) of the corals (A) *Porites cylindrica* and (B) *Acropora millepora* transplanted onto settlement blocks accessible (+H) or inaccessible (-H) to herbivores, both without (-N) and with (+N) nutrient enrichment, when deployed on a protected reef (MPA; black bars) or on an adjacent fished reef (non-MPA; white bars) for 152 d (n = 5-10 treatment^{-1} site^{-1}). *p* values are from three factor analysis of variance (ANOVA) of rank-transformed data. See Table B.3 for complete ANOVA results. Horizontal bars indicate non-significant differences between sites (S), within a treatment. Note scale differences on y-axes.

Discussion

The processes mediating large-scale shifts in coral reef community structure are debated (McCook 1996, Lapointe 1997, Hughes et al. 1999, Burkepile and Hay 2006, Littler et al. 2006a, Littler et al. 2006b, Heck and Valentine 2007, Houk et al. 2010, Smith et al. 2010), in part due to a reasonable assumption that nutrients may commonly be limiting in tropical waters and due to a few conflicting results from field experiments manipulating nutrients and herbivory. It can also be argued that several previous studies documenting strong effects of herbivory and weak effects of nutrient enrichment may have underestimated nutrient effects because studies did not run for the 3-4 months it may take for nutrient effects to appear, and/or were conducted on reefs dominated by algae instead of corals (Smith et al. 2010). However, a preponderance of rigorous field experiments suggest that herbivory plays a critical role in controlling algal community development, while nutrients play a more minor role (Burkepile and Hay 2006, Heck and Valentine 2007). Our study supports that emerging consensus; we found strong effects of herbivory and minimal effects of nutrients on algal proliferation. These effects were documented on both a coral-dominated and an algal-dominated reef, and over a duration sufficient to allow slower acting nutrient effects to emerge. On coral-dominated (MPA) and macroalgal-dominated (non-MPA) reefs, the exclusion of large herbivores significantly increased total macroalgae, upright fleshy macroalgae, and cyanobacteria cover, but nutrient addition did not stimulate cover or mass of these algae (Figures 3.1 and 3.2) and, in fact, inhibited accumulation for some algal types under reduced herbivory. Moreover, herbivory significantly enhanced the cover of CCA (some of which cue coral recruitment) and short algal turfs – both characteristic components of

healthy reefs. Nutrients had no significant effect on these algal types (Figure 3.1). Thus, between-site and between-experiment differences in nutrient effects cannot be explained consistently by benthic community composition or experiment duration alone.

Debates over the importance of top-down vs. bottom-up regulation of algal communities on coral reefs may stem, in part, from discrepancies between empirical findings and theoretical predictions. The relative dominance model (RDM) (Littler and Littler 1984, Littler et al. 2006a) predicts algal and coral community structure as a function of interactions between grazing intensity and nutrient enrichment, and suggests that turf algal communities will develop with reduced herbivory, but that elevated nutrients are required for the proliferation of upright macroalgae. A limited number of studies suggest that nutrients can drive macroalgal production in some locations (Smith et al. 2001, Lapointe et al. 2004, Littler et al. 2006a), especially when herbivores are excluded (Smith et al. 2010), but our study and the majority of other field tests (*e.g.*, McCook 1996, Miller et al. 1999, Thacker et al. 2001, Belliveau and Paul 2002, Diaz-Pulido and McCook 2003, McClanahan et al. 2003, Burkepile and Hay 2009, Sotka and Hay 2009) find limited support for the RDM. Although the RDM has been a poor predictor of most experimental outcomes, herbivory and nutrient enrichment can interact in complex ways that may vary with ecosystem productivity, latitude, algal functional group, intensity of herbivory, and duration of study - making variance between locations or times likely (Burkepile and Hay 2006, Houk et al. 2010, Smith et al. 2010).

Exclusion of large herbivores, but not nutrient enrichment, increased sediment accumulation on our experimental blocks by 49-89% (Figure 3.3). Interestingly, mean total algal cover was significantly correlated with mean total sediment load across our

treatments and sites (Spearman rank correlation: r = 0.79, p = 0.015, n = 8), suggesting a strong link between herbivory and sediment accumulation, likely by algal entrapment of sediments. Indeed, other field studies have also found a relationship between algal biomass and sediment load (Smith et al. 2001, Belliveau and Paul 2002, Stamski and Field 2006), and that sediments can strongly suppress herbivory (Bellwood and Fulton 2008) – suggesting positive feedbacks among herbivore loss, macroalgal proliferation, and sediment accumulation could reinforce phase-shifts to macroalgae. How feedbacks might vary with domination by different algal types (*e.g.*, small turfs vs. intermediate sized species like *Padina* vs. large macrophytes like *Sargassum*) has not been directly addressed, but net sediment accumulation and the strength of feedbacks might vary with stage of algal development, wave exposure, and depth (Steneck 1997). While the exclusion of herbivores increased sediments on blocks at both our MPA and non-MPA sites, net sediment loads were significantly higher in the non-MPA regardless of treatment, indicating that attributes unique to our non-MPA site (*e.g.*, decreased grazing due to fishing, riverine discharge of sediments, domination by large macroalgae) contributed to net sediment accumulation at this location.

In contrast with previous field experiments documenting that macroalgae can suppress coral growth and survivorship (Lewis 1986, Hughes et al. 2007, Burkepile and Hay 2008, Burkepile and Hay 2009), the manipulation of herbivores and nutrients in our experiment had no statistically detectable effect on growth of the corals *Porites cylindrica* or *Acropora millepora*, but the nearly significant (p = 0.075) effect of herbivores on *A. millepora* is suggestive (Figure 3.4). It should be noted that greater than 95% of upright macroalgal biomass found on our herbivore exclusion blocks was *Padina*

boryana, an alga that has little effect on *P. cylindrica* or *A. millepora* relative to several other algal species on this reef (Rasher and Hay 2010, Rasher et al. 2011). In addition, our studies started with corals transplanted to unoccupied experimental blocks; effects of macroalgae on corals would have been delayed until macrophytes had time to colonize and grow to appreciable size. Because macroalgae generally take about 3-5 months to recruit and grow to cover ≥ 20% of substrate in such experiments (Miller et al. 1999, Burkepile and Hay 2009, Smith et al. 2010), it is possible that we would have detected an effect of herbivores on corals (via increased competition from macroalgae) if our experiment had run longer (see Figure 3.4B).

Porites cylindrica and *Acropora millepora* grew significantly less on blocks deployed on a reef subject to fishing and riverine discharge vs. a protected reef. Hypotheses to explain this site difference could include effects of sediments, salinity, or abundant nearby macroalgae on coral growth. Because sediment accumulation can suppress coral growth and survivorship (Nugues and Roberts 2003, Birrell et al. 2005), and net sediment accumulation was significantly greater within the non-MPA vs. MPA, it is possible that between-site differences in net sediment accumulation contributed to differences in coral growth between MPA and non-MPA reefs (Figure 3.4). Alternatively, algal canopies and mats can produce a physio-chemical environment that is detrimental to corals, and have been reported to release water-soluble compounds that indirectly harm corals by stimulating harmful, coral-associated microbes (Smith et al. 2006, Hauri et al. 2010); thus the preponderance of macroalgae surrounding our blocks within the non-MPA (47% cover) could have negatively impacted coral growth relative to blocks deployed within the MPA (3% macroalgal cover) (Rasher and Hay 2010).

We conducted our manipulative study on geographically similar, adjacent reefs subject to either (1) fishing and riverine input or (2) protection from harvest, to assess whether herbivory, eutrophication, or the interaction of these processes differ based on human fishing practice or riverine influence. A limitation of the MPA vs. non-MPA contrast is that there is only one of each, thus potentially confounding MPA effect with location. This limitation is reduced to some extent by the sites being adjacent and by statements of villagers that the algal-dominated non-MPA site supported a coral community like that in the MPA some 30+ years ago. One might expect greater macroalgal cover on blocks accessible to herbivores within the non-MPA vs. the MPA, given (1) the potential for increased propagule supply due to surrounding high macroalgal cover (47% vs. 3% cover of macroalgae; Rasher and Hay 2010), (2) the low macroalgal grazing rates at this site (Rasher and Hay 2010), (3) the potential for terrestrially derived nutrients to increase algal growth via riverine discharge onto this reef, and/or (4) the dilution of herbivore grazing effort over increasing substrate as corals decline and are replaced by macroalgae (Mumby et al. 2007b). Yet herbivores strongly impacted algal communities even on a heavily fished reef dominated by macroalgae (Figures 3.1 and 3.2), highlighting the primacy of top-down effects on algae and their cascading impacts on reef community state (Birrell et al. 2008, Hughes et al. 2010). However, high grazing rates on open blocks within the non-MPA could have resulted from exploited herbivore species concentrating their grazing on these blocks (in preference to the surrounding natural substrate) because these herbivores prefer algae found on new substrates undergoing primary succession (such as small turfs) to large macroalgae common on older substrates in the non-MPA (Burkepile and Hay 2010). Herbivore effects can differ

dramatically on substrates supporting communities of different ages (Burkepile and Hay 2008, Burkepile and Hay 2010).

Patterns of algal community development documented here (Figures 3.1 and 3.2) suggest that the 15 times greater cover of macroalgae on natural substrates in the non-MPA compared to the MPA (Rasher and Hay 2010) is not due to nutrient stimulation of macroalgal growth in the non-MPA. When large herbivores were excluded in the presence of ambient nutrients (-H-N), macroalgae grew as well or better in the coral-dominated MPA as in the non-MPA (Figures 3.1 and 3.2), where one might expect nutrient input from the river and nearby village. Additionally, nutrient concentrations (C:N ratio) of *Padina boryana* growing on non-enriched blocks excluded from herbivores (-H-N) did not differ between reefs, suggesting similar baseline nutrient levels between sites. Although algal nutrient analyses showed that macroalgae utilized our enriched nutrient supply (see C:N ratios of enriched vs. non-enriched blocks), this did not result in increased algal cover at either site, indicating that macroalgae were not nutrient limited on either reef. Thus, the 47% macroalgal cover in the non-MPA vs. 3% cover in the MPA (Rasher and Hay 2010) appears to be from differential rates of algal removal by herbivory, not differential rates of algal growth based on nutrient supply or other differing physical regimes.

Our elemental and isotopic measurements are consistent with this top-down interpretation. The C:N ratio of *P. boryana* varied between 18.4 and 28.9, which matches the upper portion of the range reported for samples of *Padina australis* collected across a set of reefs with differing degrees of exposure to terrigenous nutrients (11.8 - 30.1; Umezawa et al. 2002). Umezawa et al. (2007) explored the controls on *Padina* C:N ratio

by incubating field-collected algae (C:N = 22) under varying conditions of light and nutrient limitation, yielding a range of about 16.5 (low light, high nutrients) to > 45 (high light, low N). In our study, C:N ratios averaged ~22-23, suggesting that ample nutrients were available for growth at both sites, and were significantly elevated within our fertilization treatment but did not result in increased macroalgal production. Moreover, our elemental composition data imply that *P. boryana* grew under conditions of neither severe nutrient limitation (*i.e.*, C:N ratio > 30) nor very high nutrient availability (C:N ratio < 15).

Our N and C isotopic data provide additional insights into the growth conditions experienced by *P. boryana* across the study area. The $\delta^{13}C$ of *Padina* tissues increases linearly with growth rate (Umezawa et al. 2007). Our data show intriguing but not significant contrasts with higher $\delta^{13}C$ values, implying higher growth rates, in the MPA than in the non-MPA, and higher $\delta^{13}C$ values for *Padina* growing on non-enriched vs. enriched blocks. The site (MPA vs. non-MPA) difference may reflect reduced competition for light, or some other non-nutrient resource, on the MPA experimental blocks because of reduced macroalgal biomass on the surrounding reef.

The above interpretation is supported by our nitrogen isotopic measurements, which provide an integrative record of the nutrient sources supporting growth (Umezawa et al. 2002, Umezawa et al. 2007). We found significantly higher $\delta^{15}N$ values for *P. boryana* collected on MPA blocks than on non-MPA blocks, but no significant $\delta^{15}N$ contrast between non-enriched and enriched blocks within study sites. The higher $\delta^{15}N$ in the MPA contrasts with previous reports of a simple relationship between terrigenous input (high $\delta^{15}N$) and algal $\delta^{15}N$ (Umezawa et al. 2007), but is consistent with a relative

lack of nutrient limitation and an isotopically uniform supply of N throughout the study area. In this scenario, variation in the $\delta^{15}N$ of macroalgae is driven by isotopic fractionation and reflects a greater fractional consumption of nutrients in the MPA than in the non-MPA, perhaps because of the higher terrigenous inputs to the non-MPA.

Emerging research suggests the human harvest of marine herbivores plays a pivotal role in reef decline (Lewis 1986, Jackson et al. 2001, Bellwood et al. 2004, Mumby and Steneck 2008, Hughes et al. 2010) by compromising processes that facilitate coral recovery from, and resistance to, a range of disturbances (Hughes et al. 2007, Mumby et al. 2007a, Mumby et al. 2007b). Indeed, our study and numerous other recent field experiments (*e.g.*, Belliveau and Paul 2002, Diaz-Pulido and McCook 2003, Burkepile and Hay 2009, Sotka and Hay 2009) indicate that herbivores limit the establishment of algae (Figure 3.1), limit sediment accumulation (Figure 3.3), and promote the establishment of CCA (Figure 3.1), all of which are critical to successful coral recruitment and/or growth following disturbance (Birrell et al. 2008). These critical ecological processes are reduced or lost with the removal of functionally important herbivores, and the impacts of their loss may be magnified by nutrient enrichment (Burkepile and Hay 2006, Smith et al. 2010). Prioritization of management approaches that protect critical processes such as herbivory that bolster coral reefs against phase-shifts to macroalgae should slow reef decline, and facilitate coral recovery from the numerous stresses impacting present-day reefs (Knowlton and Jackson 2008, Carilli et al. 2009, Mumby and Harborne 2010, Selig and Bruno 2010).

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CHAPTER 4

CONSUMER DIVERSITY INTERACTS WITH PREY DEFENSES TO DRIVE ECOSYSTEM FUNCTION

Abstract

Prey traits linking consumer diversity to ecosystem functioning remain poorly understood. On tropical reefs, herbivores promote coral resilience by suppressing competing macroalgae, but the roles of herbivore diversity, macroalgal defenses, and their interactions in affecting reef resilience and function are unclear. We transplanted, from degraded to protected reefs, seven macroalgae that differed in allelopathy against corals and assessed the functional redundancy vs. complementarity of herbivorous fishes consuming these macroalgae. Surprisingly, of the 25+ species of herbivorous fishes on the reef, 97% of macroalgal consumption could be attributed to just four fishes – the unicornfishes *Naso lituratus* and *Naso unicornis*, the parrotfish *Chlorurus sordidus*, and the rabbitfish *Siganus argenteus*. Fishes exhibited complementary feeding, with the most allelopathic algae each being consumed by only one fish. Field bioassays revealed that complementary feeding among herbivores was driven largely by differential herbivore tolerance to algal chemical defenses. Moreover, the suite of herbivores scraping or excavating the substratum, and thus suppressing the establishment of late-succession macroalgae, differed fundamentally from those fishes removing established macroalgae. Thus, the total diet breadth of the herbivore community and the probability of all macroalgae being suppressed by herbivores increased as a function of increasing herbivore species richness. Interactions between algal defenses and herbivore tolerances

makes consumer diversity essential for preserving the ecosystem functioning and resilience of coral reefs.

Introduction

Biodiversity promotes the functioning, stability, and productivity of ecosystems, as well as their services to human societies (Balvanera et al. 2006, Cardinale et al. 2006, Worm et al. 2006). Positive effects of biodiversity on ecosystem functioning may occur due to the increasing probability of including a particular species with a disproportionately large impact (the selection effect), or to the inclusion of multiple species with complementary and additive impacts (the complementarity effect), on ecosystems processes as communities increase in species richness (Loreau and Hector 2001). As such, elucidation of the functional roles of species in natural communities is critical for understanding links between biodiversity and ecosystem functioning, and for determining if species-specific or diversity-oriented management approaches are most effective for maintaining ecosystem processes (Duffy 2009, O'Gorman et al. 2011). Our present understanding of the outcomes and mechanisms of diversity effects on ecosystems generally come from small-scale experiments utilizing a relatively limited number of species, and have historically focused on organisms from lower trophic levels (Balvanera et al. 2006, Duffy et al. 2007). Thus, the functional range of consumers and effects of consumer diversity on ecosystem processes remain poorly understood in many natural communities (Duffy 2002, Balvanera et al. 2006).

Consumers have cascading effects on the structure and function of terrestrial, aquatic, and marine ecosystems (Estes et al. 2011). On coral reefs, intense grazing by herbivores can remove > 90% of daily primary production (Hatcher and Larkum 1983,

Carpenter 1986), preventing the proliferation of competitively superior macroalgae that limit coral survival, growth, and reproduction (Birrell et al. 2008, Hughes et al. 2010). Consumer suppression of macroalgae thus facilitates coral recovery following disturbances, promoting the resilience of these foundation species (Hughes et al. 2010). Such dramatic top-down pressure on coral reefs has selected for macroalgae that produce a wide range of chemical and structural defenses, and in turn, for counter-adaptations by some consumers to tolerate these defenses (Schupp and Paul 1994, Hay 1997). Yet, the ecological consequences of consumer-prey co-adaptations for coral reef structure and function are not well understood. Interactions between consumer and prey functional traits that link top-down control of ecosystems to consumer diversity effects on ecosystem function remain unclear (Hillebrand and Cardinale 2004, Ives et al. 2005, Duffy et al. 2007).

Coral reef herbivores can be broadly classified into functional groups of (a) substratum grazers and excavators (*i.e.*, species that consume the epilithic algal matrix from the substratum, thereby keeping the community in an early successional stage largely devoid of upright macroalgae) and (b) macroalgal browsers (*i.e.*, species that remove large, established macroalgae) (Bellwood et al. 2004, Burkepile and Hay 2010). However, the herbivores comprising each of these functional classes span a range of taxonomic groups, and can differ in ecological function despite being similar morphologically, taxonomically, and sometimes physiologically, making generalized predictions of functional roles based on these characteristics problematic (Choat et al. 2002, Burkepile and Hay 2008, Fox et al. 2009).

Recent field studies have elucidated the functional roles of some macroalgal browsers (*e.g.*, Burkepile and Hay 2008, Hoey and Bellwood 2009), but few studies have examined herbivore browsing responses to a diverse array of macroalgae that commonly characterize degraded reefs (Mantyka and Bellwood 2007a, Mantyka and Bellwood 2007b, Burkepile and Hay 2008), limiting our ability to detect complementarity among macroalgal browsers or to understand how differing algal traits might drive consumer complementarity. Consumer complementarity documented in marine soft sediment (Duffy et al. 2003), terrestrial shrub (Rogosic et al. 2006), and aquatic rocky reef communities (Duponchelle et al. 2005) suggests that complementarity could be an important mechanism linking consumer diversity to ecosystem functioning in numerous ecosystems, but even when complementarity is demonstrated the consumer or prey traits producing the pattern are rarely understood (Byrnes et al. 2006).

On many tropical reefs experiencing coral decline from anthropogenic disturbance, the loss of consumers to overfishing has resulted in failure of top-down control, triggering phase-shifts from coral toward increased cover of macroalgae (Hughes et al. 2010). Abundant macroalgae limit coral survival and recruitment by competition (Hughes et al. 2007, Diaz-Pulido et al. 2010, Rasher and Hay 2010), thereby forming ecological feedbacks that further limit coral recovery and reinforce the dominance of macroalgae (Mumby and Steneck 2008, Hughes et al. 2010). However, the mechanisms and outcomes of algal-coral competition on degraded reefs are species-specific, and vary in part due to the unique traits of algal and coral species (Rasher et al. 2011). Our current knowledge of herbivory and competition on coral reefs suggests that a clearer understanding of the functional roles of herbivores as algal browsers, of macroalgae as

coral competitors, and of macroalgae as herbivore prey is needed for effective management of reef resilience (Bellwood et al. 2004, Hughes et al. 2010).

Here we determined (1) the extent to which herbivory suppresses an array of common macroalgae that have variable effects on coral fitness, (2) the functional identities and redundancies of consumers capable of removing these macroalgae vs. grazing the epilithic algal matrix on the substratum, (3) how consumer tolerances to prey chemical defenses affects browser complementarity, and thus (4) how consumer diversity and prey defenses interact to affect the critical process of herbivory on coral reefs.

Materials and Methods

Study site characteristics

Feeding assays were conducted in May-June 2010 and 2011 on three reef flats within no-take marine reserves at Namada, Vatu-o-lailai, and Votua villages along the Coral Coast of Viti Levu, Fiji. Reserves are located along an 11 Km continuous stretch of fringing reef, and are separated by 3.3-7.6 Km. Established in 2002-3 (*i.e.*, 8-9 years before our study), these reserves are characterized by high coral cover (45-57%, predominantly massive *Porites* and a diverse assemblage of *Acropora* spp.), low macroalgal cover (0-3%), and high biomass of herbivorous fishes (Simpson 2009; Table 4.1). Between reserves, sections of the reef flat are open to artisanal fishing at all trophic levels (Simpson 2009); these adjacent fished areas ("non-reserves") are characterized by low biomass of herbivorous fishes (Simpson 2009), high macroalgal cover (6-47%), and low coral cover (3-19%) (Table 4.1).

In June 2009, cover of macroalgae and hard corals on reserve and non-reserve reefs at each village was assessed using 30 m point-intercept transects ($n = 10$ reef^{-1}

location^{-1}). We deployed the first transect in the middle of each site along a randomly generated compass bearing. Subsequent transects were run parallel to this initial transect at randomly assigned intervals of 2-10 m. Macroalgal and hard coral presence was assessed at each 1m interval along each transect (*i.e.*, 300 points site^{-1}).

Macroalgal consumption by herbivores

To determine the susceptibility of macroalgae to herbivore removal, we collected seven common macroalgae from non-reserve reefs (the brown algae *Sargassum polycystum*, *Turbinaria conoides*, *Padina boryana*, and *Dictyota bartayresiana,* the red algae *Amphiroa crassa* and *Galaxaura filamentosa*, and the green alga *Chlorodesmis fastigiata*), deployed them within the three no-take reserves, and assessed loss of mass relative to caged controls over 48 h. We used these macroalgae because they: (1) encompass a range of taxonomic, morphological, and functional forms, (2) are common in the non-reserves, and (3) show a broad range of competitive impacts on corals in Fiji (Rasher and Hay 2010, Rasher et al. 2011) and on other reefs in the South Pacific (Hughes et al. 2007, Diaz-Pulido et al. 2010).

Algae were collected from non-reserve habitats at Votua village. We removed excess water using a salad spinner (10 revolutions), selected thalli of each species to roughly standardize apparency within and between species, weighed each alga, and arranged one thallus of each of the seven algal species in random order ~7 cm apart on a 60 cm section of three-stranded nylon rope. Paired treatment (feeding allowed) and control (caged) ropes were assembled in the same manner (n = 12 pairs reserve^{-1}). Standardizing apparency generated initial masses (grams, mean ± SE) of: *S. polycystum* (2.35 ± 0.08), *T. conoides* (3.50 ± 0.11), *P. boryana* (2.36 ± 0.09), *D. bartayresiana* (6.13

± 0.16), *C. fastigiata* (4.55 ± 0.21), *G. filamentosa* (1.60 ± 0.07), and *A. crassa* (0.90 ± 0.04).

We deployed paired treatment and control ropes in interconnected networks of reef flat pools accessible to herbivores during both low (~1.5 m depth) and high (~2.5 m depth) tidal periods. Treatment ropes were attached to dead coral fragments and deployed on the substratum at 5-7 m intervals. Each paired control was deployed in a cage (65 cm long by 10 cm tall and wide) made of wire mesh (1 cm^2 grid), and placed within 1 m of its paired treatment rope. Deployment was during calm conditions, with minimal surge. After 48 h, we bagged ropes *in situ*, returned them to the lab, and spun and weighed each alga as described above. The mass of each alga consumed was calculated using the formula: $[T_i \times (C_f / C_i)]-T_f$, where T_i and T_f were the initial and final masses (respectively) of a treatment alga exposed to herbivory, and C_i and C_f were the initial and final masses (respectively) of its paired control excluded from herbivory. Percentage of each alga consumed was calculated to facilitate comparisons among species.

Identification of herbivore functional roles

To determine the identity of herbivores consuming macroalgae, we deployed the same seven algae in front of remote video cameras. Assays were deployed within each of the three reserves between 0800-1400 h, during low tide. For each assay, we deployed three individual thalli of each algal species in a conspecific group (roughly standardizing visual apparency within and between species), and configured conspecific groups randomly among four parallel 60 cm ropes, held flush to the substrate with steel bars. Tripods with cameras and ropes with algae were deployed for three consecutive days

prior to the experiment to acclimatize fish to the presence of the experimental equipment and macroalgae.

Feeding assays (n = 3 reserve^{-1}) were deployed within a quadrat (1 m^2) and filmed for 1 h. We repeated each 1 h feeding assay at the same locations in each reserve over five consecutive days to capture effects of roving herbivores that fluctuate in space and time. This produced 15 feeding trials reserve^{-1} (3 locations reserve^{-1} x 5 d = 45 h of video). Assays were conducted sequentially across the three reserves over the course of a three-week period.

Videoed feeding observations were assessed for nominally herbivorous fishes (*sensu* Choat et al. 2002), including members of the families Labridae (parrotfishes), Acanthuridae, Siganidae, and Kyphosidae. Juvenile fishes (< 10 cm) and pomacentrids were not scored due to the difficulty of accurately assessing their impacts on the macroalgae. For each visit by an herbivore in the 1 m^2 quadrat, we recorded its species and size, and scored the number of bites it took from each deployed macroalga and from the epilithic algal matrix growing on the substratum within the 1 m^2 area. If multiple rapid bites from an herbivore were not discernable they were treated as one bite, but this occurred infrequently. Scoring was terminated when ~75% of any macroalgal species was removed; at this point, the relative availability of each algal species was unacceptably skewed and could impact the relative preferences of herbivores. Because most assays lasted less than 30 min before some algal species was 75% removed, only the first 30 min of each video was analyzed.

To correct for size-dependent browsing and determine the relative impact of each fish species on each alga, we converted individual bites to mass-standardized bites (Hoey

and Bellwood 2009) using published length-biomass relationships (Kulbicki et al. 2005). Mass standardized bites were calculated as the product of [# of bites] and [biomass (kg)] of the fish.

Algal chemical defense assays

To assess the role of algal chemical defenses in affecting complimentary feeding among herbivores, we extracted the hydrophobic chemicals from algae avoided by herbivores, and coated these extracts onto algae targeted by those herbivores (Meyer et al. 1994). We exhaustively extracted *C. fastigiata*, *A. crassa*, *G. filamentosa*, and *D. bartayresiana* with 100% methanol, dried each extract by rotary evaporation, and partitioned each extract between water and ethyl acetate. The hydrophobic (ethyl acetate) fraction of each extract was retained, dried by rotary evaporation, and stored at -5^{o}C until used in feeding assays.

The unicornfishes *Naso lituratus* and *Naso unicornis* did not consume the green alga *C. fastigiata* or the red algae *A. crassa* and *G. filamentosa*, but readily consumed the brown alga *P. boryana*. To test if the avoided algae possessed chemical defenses against *N. lituratus* and *N. unicornis*, we suspended each algal extract in ether, coated a natural volumetric concentration of the extract on five blades (a 2.04 ± 0.03 mL volumetric equivalent, mean ± SE, n = 10) of blotted and pre-weighed *P. boryana*, allowed the ether to evaporate, and inserted these blades, each 5 cm apart, on a 60 cm section of three-stranded rope (n = 15 ropes extract^{-1}). Paired control ropes were assembled in the same manner, but *P. boryana* was coated with ether alone.

The parrotfish *Chlorurus sordidus* did not consume the brown alga *D. bartayresiana* or the green alga *C. fastigiata*, and consumed little of the red alga *G.*

fastigiata. To test if these algae were chemically defended against *C. sordidus*, we suspended each algal extract in ether, coated the extract at natural volumetric concentration onto five (a 1.23 ± 0.03 mL volumetric equivalent, mean ± SE, n = 10) blotted and pre-weighed branches of *A. crassa* (a preferred prey of *C. sordidus*, but not of other fishes), allowed the ether to evaporate, and constructed ropes holding treatment and control blades as described above (n = 15 pairs extract^{-1}). Assays to test the tolerance of *Siganus argenteus* to algal chemical defenses could not be conducted, because the only alga it consumed (*C. fastigiata*) was filamentous and held too much water when blotted for our hydrophobic extracts to effectively adhere to its surfaces.

Assays involving coated *P. boryana* were conducted within Votua's marine reserve, during low tides (~2 m depth) between 0800-1400 h, over three days (*i.e.*, one algal extract day^{-1}). Paired treatment and control ropes were deployed 0.5-0.75 m apart on a small coral colony on the substratum. Because feeding on *P. boryana* was rapid (~10 min pair^{-1}), treatment and control ropes were deployed one pair at a time, monitored, and recollected when approximately 50% of the total algal mass (treatment and control combined) was consumed. Subsequent replicates were deployed 2-5 m from the previous location (n = 15 pairs extract^{-1}). Post-assay ropes were bagged in the field, and blotted and re-weighed at the laboratory to determine the mass of treatment vs. control algae consumed. Given the short duration of each assay and our visual assessment that algal portions were not lost to processes other than herbivory, caged controls were not deployed. We observed browsing by only *Naso lituratus* and *Naso unicornis*.

Due to lower browsing rates on *A. crassa*, we deployed paired *A. crassa* treatment and control ropes in Votua's marine reserve for 24 h. Pairs were deployed within 0.5-0.75 m of each other, with pairs separated by 5-7 m. Caged controls were not deployed, as we assumed non-browsing losses to paired treatment and control algae would be similar, and assays were deployed during calm conditions. Ropes were re-collected, blotted, and re-weighed as above.

Statistical analysis

When multiple macroalgae (*i.e.*, treatments) are simultaneously offered to herbivores in multiple-choice assays the treatments may not be independent, thus violating the assumptions of ANOVA procedures (Roa 1992). We therefore analyzed our field herbivory assays using non-parametric Friedman's tests (Roa 1992, Mantyka and Bellwood 2007b). Significant differences were further evaluated using Friedman's post-hoc multiple comparisons tests.

For each video assay, we summed the mass-standardized bites for each fish species on each alga and scaled them to rates h^{-1}. Because videoed feeding assays (n = 3 $reserve^{-1}$ day^{-1}) were conducted in the same physical locations within each reserve on sequential days, multiple assays at each feeding station within a reserve may not be independent. We therefore averaged results from each feeding station over the five days. Independent feeding station averages were then pooled across the three reserves (n = 3 $reserve^{-1}$) for analysis. Friedman's tests were conducted for each of the four dominant browsing herbivores; these fishes accounted for 97% of all bites on macroalgae. Grazing rates for the four dominant fishes that fed primarily from the epilithic algal matrix were also evaluated using Friedman's tests.

Diet breadth was evaluated for each of the eight dominant herbivores. For each, we calculated Levins' *B* using the proportions of their total bites taken on each of the eight algal resources (seven macroalgae and the natural epilithic algal matrix) in our 45 videoed assays. We then calculated the total diet breadths of herbivore communities of differing species richness, assembled at random from the eight dominant herbivores that fed in our assays. At each level of richness, we used either all possible species combinations (at levels 1, 7 & 8 of consumer species richness) or we randomly selected eight unique species combinations from all possible combinations (levels 2-6), and calculated the diet breadth for each multi-species combination (*i.e.*, summing bite count data for all fish in a combination and calculating Levins' *B*).

As data for assays assessing effects of algal extracts on herbivore feeding could not be transformed to meet the assumptions of paired t tests, we evaluated these data with non-parametric Quade's tests (Roa 1992). Friedman's and Quade's tests were performed using the program "R" (version 2.13.2; R Development Core Team 2011) and Friedman's post-hoc analyses were conducted using the R package "agricolae" (v. 1.0-9; de Mendiburu 2010).

Results

Macroalgal consumption by herbivores

In reserves, the brown algae *Sargassum polycystum*, *Turbinaria conoides*, *Padina boryana*, and *Dictyota bartayresiana* were rapidly consumed (85-99% 48 h^{-1}) by herbivorous fishes, while the green alga *Chlorodesmis fastigiata* and the red algae *Galaxaura filamentosa* and *Amphiroa crassa* were consumed at appreciable, but significantly lower rates (4-57% 48 h^{-1}; Figure 4.1). Reflective of these high browsing

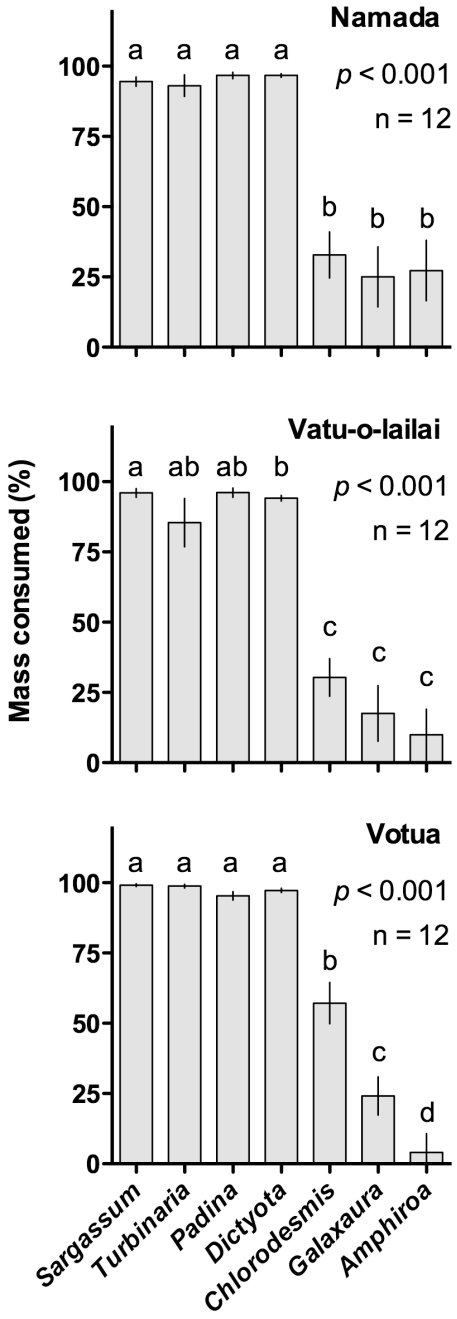

Figure 4.1: Macroalgal removal (% 48 h^{-1}; mean ± SE) by herbivorous fishes when macroalgae common to degraded reefs were deployed in no-take marine reserves at Namada, Vatu-o-lailai, and Votua villages. Analysis by Friedman's tests and post-hoc comparisons. Letters indicate significant groupings. n = 12 $reserve^{-1}$.

rates, macroalgal cover was only 0-3% in reserves. In contrast, macroalgal cover was 6-47% in adjacent non-reserves subject to human activity (Table 4.1). Coral cover was 2-19x higher in reserves (45-57%) than in non-reserves (3-19%).

Table 4.1: Percentage coral and macroalgal cover (mean ± SE) inside and outside of marine reserves at Namada, Vatu-o-lailai, and Votua villages (n = 10 reef^{-1} location^{-1}).

Location	Benthos	Cover (mean % ± SE)	
		Reserve	Non-reserve
Namada	Macroalgae	0.0 ± 0.0	5.7 ± 1.3
	Hard coral	44.7 ± 2.3	19.3 ± 2.1
Vatu-o-lailai	Macroalgae	0.3 ± 0.3	43.0 ± 4.3
	Hard coral	45.3 ± 6.2	16.7 ± 1.9
Votua	Macroalgae	2.7 ± 1.1	47.3 ± 4.9
	Hard coral	57.3 ± 2.6	3.0 ± 1.7

Identities of macroalgal browsers vs. substratum grazers

When seven common macroalgae were deployed and videoed (n = 3 reserve^{-1}) over five consecutive days within each of the no-take reserves, we quantified 19,757 fish bites on these macroalgae. Four fishes were responsible for 97% of all bites – the unicornfishes *Naso lituratus* and *Naso unicornis*, the parrotfish *Chlorurus sordidus*, and the rabbitfish *Siganus argenteus*. *N. lituratus* and *N. unicornis* both concentrated their feeding on the brown macroalgae *S. polycystum*, *T. conoides*, *P. boryana*, and *D. bartayresiana*, but relative feeding rates among brown macroalgae differed somewhat between these fishes (Figure 4.2A & B). Only initial phase (IP) *C. sordidus* fed on the red algae *A. crassa* or *G. filamentosa*; it also consumed *S. polycystum* but at modest rates

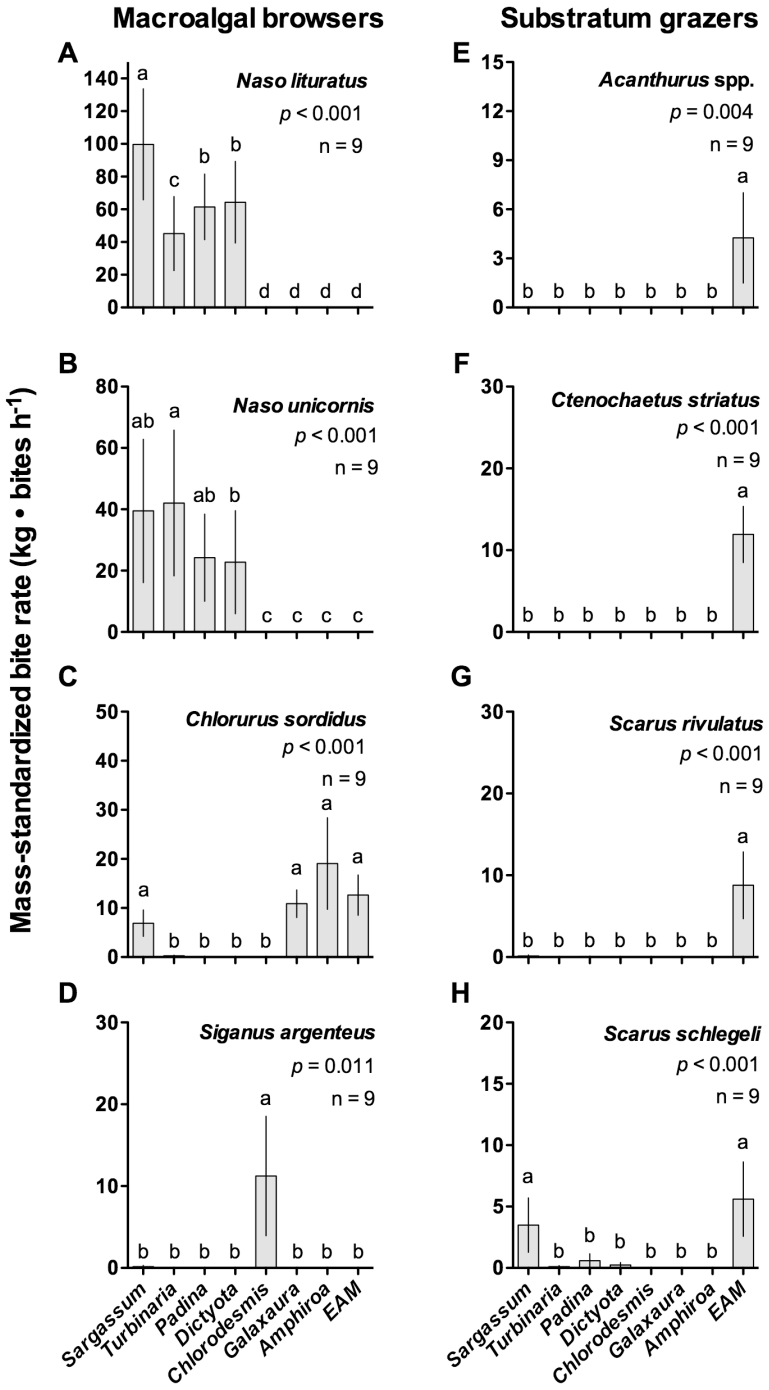

Figure 4.2: Mass-standardized bite rates (kg • bites h^{-1}; mean ± SE) of (A-D) dominant browsers on seven macroalgae common to degraded reef habitats, or of (E-H) dominant grazers on the epilithic algal matrix (EAM) growing on the substratum. Data for each consumer were analyzed separately by a Friedman's test and post-hoc comparison. Letters indicate significant groupings. Scarids were all initial phase (IP). Note scale differences between y-axes. n = 9.

relative to both *Naso* species (Figure 4.2C). Only *S. argenteus* consumed the green alga *Chlorodesmis fastigiata*, and it consumed no other alga (Figure 4.2D).

With the exception of *C. sordidus*, the major consumers of macroalgae (Figure 4.2A-D) did not graze the epilithic algal matrix. However, we observed other fishes that took 4,999 bites from the epilithic algal matrix. Similar to patterns of macroalgal browsing, 98% of all bites were by only five fishes - the parrotfishes *C. sordidus* (IP), *Scarus rivulatus* (IP), and *Scarus schlegeli* (IP), and the surgeonfishes *Ctenochaetus striatus* and *Acanthurus spp.* (cf. *nigricauda*) (Figure 4.2E-H). *S. rivulatu*s, *C. striatus*, and *Acanthurus* spp. grazed the epilithic algal matrix almost exclusively, and in preference to all macroalgae (Figure 4.2E-G). *S. schlegeli* fed from both *S. polycystum* (or its epiphytes) and the epilithic algal matrix at low rates (Figure 4.2H), while *C. sordidus* grazed the epilithic algal matrix, *A. crassa*, *G. filamentosa*, and *S. polycystum* at similar rates (Figure 4.2C).

Herbivores exhibited strong feeding complementarity within function groups (macroalgal browsers) and between functional groups (browsers vs. scrapers), allowing no algal resources to escape attack from all consumers (Figure 4.2). Consistent with this notion, the potential breadth of algal resources utilized by randomly assembled mixes of these herbivores increased as a function of increasing herbivore species richness (Figure 4.3).

Algal chemical defenses

When hydrophobic extracts of *C. fastigiata* and *G. filamentosa* were coated onto *P. boryana* (a preferred prey of both *Naso* species) at natural concentration, both extracts significantly suppressed *Naso* browsing, relative to *P. boryana* coated with solvent alone

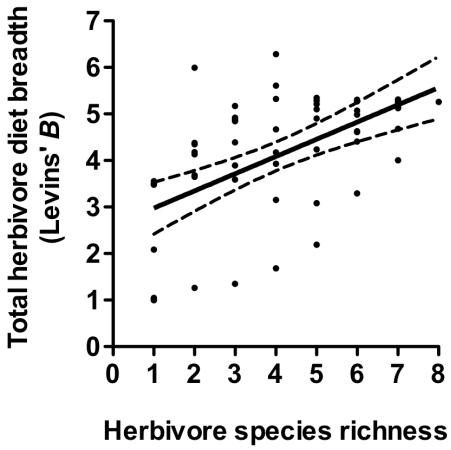

Figure 4.3: Diet breadth (Levins' *B*) of the reef herbivore community as a function of herbivore species richness. At each level of species richness, we plotted all species combinations (levels 1, 7-8) or eight randomly determined combinations from the many available (levels 2-6). The solid line represents a linear regression. The dotted lines show 95% confidence intervals of the regression.

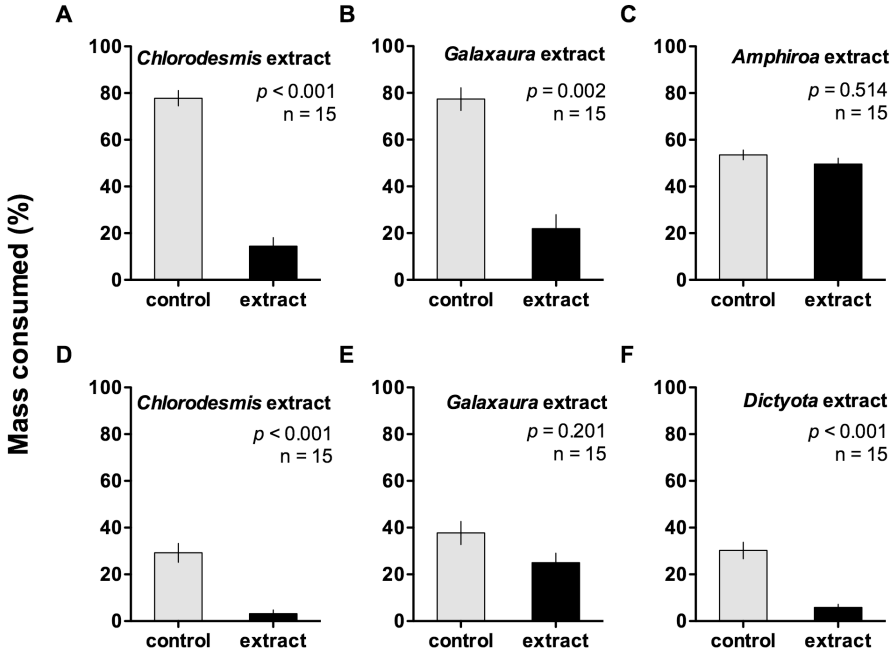

Figure 4.4: (A-C) Percent mass (mean ± SE) of *Padina boryana* consumed by the fishes *Naso lituratus* and *Naso unicornis*, or (D-F) percent mass (mean ± SE) of *Amphiroa crassa* consumed (likely by *Chlorurus sordidus*), when coated with hydrophobic extracts of algae avoided by each respective herbivore vs. paired control algae coated only with solvent. Analyzed by Quade's tests. n = 15 extract^{-1}.

($p \leq 0.002$; Figure 4.4A & B). In contrast, the extract of the avoided and heavily calcified *Amphiroa crassa* did not suppress *Naso* browsing ($p = 0.51$; Figure 4.4C).

At natural concentrations, the hydrophobic extracts of *C. fastigiata* and *D. bartayresiana* deterred fish feeding on *A. crassa* (a preferred prey of *C. sordidus*; $p < 0.001$; Figure 4.4D & F). In contrast, the extract of *G. filamentosa* (an alga consumed by *C. sordidus*, but at low rates) did not significantly suppress browsing ($p = 0.20$) despite mean grazing declining by ~34% (Figure 4.4E).

Discussion

Complementarity in consumer tolerances to prey defense could be a mechanism connecting consumer diversity with ecosystem function in many ecosystems (Hillebrand and Cardinale 2004), but there are limited data addressing this possibility in natural field settings (Duffy 2002, Ives et al. 2005, Duffy et al. 2007). On tropical reefs, we found that functional complementarity between browsing herbivores (Figure 4.2) produces a strong role for herbivore diversity *per se* in preserving ecosystem function. More species-rich assemblages of herbivores with complementary feeding strategies possess a greater cumulative diet breadth (Figure 4.3) and can thus better suppress diverse communities of macroalgae that vary in defensive strategies (Figure 4.4) and impacts on corals (Rasher et al. 2011). Our results demonstrate (1) the critical roles that consumer diversity and functional complementarity play in promoting a process (herbivory) necessary to the function of coral reefs, and (2) that *interactions* between prey defenses and consumer tolerances create this effect of biodiversity on ecosystem function.

Brown macroalgae in the genera *Sargassum*, *Turbinaria*, *Padina*, and *Dictyota* are pan-tropical and commonly proliferate on tropical reefs following the removal of key

consumers (Hughes et al. 2007, Burkepile and Hay 2008). Once established, *Sargassum* can limit the recruitment of corals (Hughes et al. 2007), and *Dictyota bartayresiana* can cause coral bleaching and mortality through allelopathic competition (Rasher and Hay 2010, Rasher et al. 2011), suggesting that dominance by these algae could limit reef recovery. For many reef systems, the identities of consumers capable of removing macroalgae, and thus reversing phase-shifts, have remained elusive. However, we found that these brown macroalgae (including the chemically rich and allelopathic *D. bartayresiana*) were rapidly and almost exclusively consumed by the unicornfishes *Naso lituratus* and *N. unicornis* (Figure 4.2). Given the significant feeding impact of these fishes on brown algae (Choat et al. 2002, Hoey and Bellwood 2009, Hoey and Bellwood 2010) that suppress coral recovery (Hughes et al. 2007), our results strengthen the notion that targeted management of *N. lituratus* and *N. unicornis* might facilitate the reversal of phase-shifts and improve coral resilience on degraded Pacific reefs.

Galaxaura filamentosa and *Chlorodesmis fastigiata* are two of the most allelopathic macroalgae on Pacific reefs, and *Amphiroa crassa* is variably allelopathic, harming some corals but not others (Rasher and Hay 2010, Rasher et al. 2011). Each of these algae were consumed by only one herbivore (Figure 4.2): *G. filamentosa* and *A. crassa* by the parrotfish *Chlorurus sordidus* (Figure 4.2C), and *C. fastigiata* by the rabbitfish *Siganus argenteus* (Figure 4.2D). Given that macroalgae of varying palatability (Figure 4.1) and allelopathic potency (Rasher *et al.* 2011) were each consumed by different herbivores (Figure 4.2), our results indicate that both individual species (Hoey and Bellwood 2009, Hoey and Bellwood 2010) and appropriate mixes of

consumers (Burkepile and Hay 2008, Burkepile and Hay 2010) are critical for preserving herbivory as a process that promotes coral reef resilience.

Complementary feeding among four fishes resulted in the consumption of all common macroalgae (Figures 4.1 and 4.2), including algae that facilitate phase-shifts by chemically suppressing coral survival and recruitment (Diaz-Pulido et al. 2010, Rasher and Hay 2010, Rasher et al. 2011) and by chemically deterring browsing by some herbivores (Schupp and Paul 1994). This complementarity effect was driven, in large part, by differential tolerances to algal chemical defenses among herbivores (Figure 4.4). Laboratory assays support our field studies, demonstrating that differential tolerances to chemical and mineral defenses may be common among reef herbivores (Paul et al. 1990, Hay et al. 1994, Meyer et al.1994, Schupp and Paul 1994). In our study, avoidance of *A. crassa* by both *Naso* species was not explained by defensive chemistry alone (Figure 4.4C), but is likely due to the heavy calcification of *A. crassa*, which could serve as a structural defense against some consumers, or as a defense that buffers the acidic gut that several surgeonfish depend on for digestion of algal tissues (Hay et al. 1994, Schupp and Paul 1994). Comparative studies of herbivore gut contents further indicate that gut physiology may play an important role in diet partitioning among reef herbivores (Choat et al. 2002, Choat et al. 2004). Our findings of complementary feeding among browsers in Fiji are consistent with patterns observed in the Caribbean Sea (Burkepile and Hay 2008) and on the Great Barrier Reef (Mantyka and Bellwood 2007a, Mantyka and Bellwood 2007b), indicating that complementarity among reef herbivores may be common.

In addition to browsers of macroalgae, we also documented five species of herbivores grazing the epilithic algal matrix growing on the substratum (Figure 4.2). *C. sordidus* and *S. schlegeli* fed on both the epilithic algal matrix and some macroalgae, but the other dominant herbivores fed only from the epilithic algal matrix or only from macroalgae (Figure 4.2). For fishes feeding on the epilithic algal matrix, differentiation of bites between small algal turfs, crustose coralline algae, sediments, detritus, etc. could not be determined due to the mixture of these foods within the algal matrix and due to the resolution of our video assays. Thus, complementarity vs. redundancy *within* this functional group could not be assessed.

Due to logistical constraints, investigators must often select a sub-set of species from a regional species pool to utilize in manipulative experiments of diversity and ecosystem functioning. While such approaches enable researchers to rigorously test the effects of diversity on an ecosystem attribute, they inhibit the detection of selection and complementarity effects that could exist within more diverse natural assemblages; these mechanisms may only be revealed at high levels of species richness that are logistically challenging to create in experiments manipulating consumer diversity (Duffy et al. 2003). By observing natural herbivore assemblages, we attempted to document the functional range of consumers in this natural community, and thus maximize our potential to identify complementarity vs. redundancy among the key species responsible for macroalgal removal. Such approaches complement manipulative studies of biodiversity-ecosystem functioning, and may also provide information on important species to select from the regional species pool for manipulative experiments.

Our results strengthen the notion that one group of coral reef herbivores prevents the establishment of macroalgae by scraping or excavating the epilithic algal matrix, and that a second group initiates the reversal of phase shifts by consuming established macroalgae (Bellwood et al. 2004, Burkepile and Hay 2010). Among this latter group, complementary feeding based on differing tolerances for algal defensive traits appears critical for producing the effect of herbivore diversity on ecosystem function (Figure 4.3); the total proportion of the algal community consumed increased directly with herbivore species richness. A diverse group of consumers is needed to control the broad range of differentially defended macroalgae that occur on degraded reefs (Figure 4.3); additionally, specific herbivores are critical for controlling some of the most allelopathic algae (Figure 4.2). The extreme variance in effects of herbivore diversity on total diet breadth that we documented for combinations of 1-5 herbivores (Figure 4.3) could explain why some reefs have failed to recover from phase-shifts to macroalgae, despite years to decades of protection (Ledlie et al. 2007, Huntington et al. 2011, but see Mumby and Harborne 2010). If re-establishing herbivore communities contain functionally similar herbivores with limited diet breadths, they will be unlikely to control a diverse array of algae possessing varied defensive traits (Figure 4.3). For example, a community of four herbivore species can produce a Levins' *B* value as low as 1.7 or as high as 6.3 depending on the vagaries of which herbivores recruit (Figure 4.3). Thus, if management efforts are to increase reef resilience, protected reefs must possess the right species mix, density, and biomass of herbivores to both limit algal proliferation on substrates not already occupied by macroalgae (Paddack et al. 2006) and remove an array of chemically and structurally defended macroalgae already on the reef (Cheal et al. 2010).

Links between changing diversity and ecosystem function remain a contentious issue, in part due to the limited number of studies addressing such questions at ecologically relevant scales (Duffy 2009). Consumers impact ecosystem function through top-down forcing (Ives et al. 2005, Duffy et al. 2007), and consumers are likely more vulnerable to localized extinction than primary producers (Duffy 2003), yet our understanding of how consumer diversity impacts ecosystem function is limited. Our study demonstrates the importance of consumer diversity and feeding complementarity to the critical ecosystem process of herbivory on a coral reef, but also reveals a strikingly limited redundancy of consumers feeding within a diverse natural assemblage. Such findings have important conservation implications, because critical ecosystem processes can rapidly deteriorate even when only one, or a few, key consumers are locally extirpated (Bellwood et al. 2011, Estes et al. 2011, O'Gorman et al. 2011). Improved knowledge of the functional roles of consumers, the prey traits affecting consumer choices, and the *interaction* of prey defenses and consumer tolerances appears critical for informed management to maintain the structure and function of natural ecosystems.

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CHAPTER 5

COMPETITION WITH CORALS INDUCES SEAWEED ALLELOCHEMICALS BUT SUPPRESSES HERBIVORE DETERRENTS

Abstract

Plants commonly induce chemical defenses in response to herbivory, but induction of chemicals to suppress competitors (allelopathy) is less well studied, and is unknown for macroalgae. Here we evaluated the inducible responses of an allelopathic and non-allelopathic macroalga to competition with a common coral, and evaluated whether these algae possessed synergies or trade-offs in the production of molecules that deter competitors vs. herbivores. When placed in contact with the coral *Porites cylindrica* vs. only the skeleton of this coral (to control for abrasion and shading), the allelopathic alga *Galaxaura filamentosa* induced allelochemicals and became more damaging to the coral, but this also resulted in a reduction of chemical defenses against reef herbivores. In contrast the non-allelopathic alga *Sargassum polycystum* did not respond chemically to algal-coral competition and incurred no change in its palatability to reef herbivores. Field bioassays using fractions of the organic extract from *G. filamentosa* demonstrated that the alga produced different molecules to deter competitors vs. herbivores; thus the field pattern of increased palatability with increased allelopathy for *G. filamentosa* suggests a trade-off between the productions of molecules that deter competitors vs. herbivores. Our findings indicate that inducible responses of marine producers to multiple ecological pressures can involve complex trade-offs, and highlight

the need to consider greater ecological complexity when investigating the ecology and evolution of chemical responses to natural enemies.

Introduction

Many organisms induce defenses against natural enemies following initial attack, or in response to the attack of a nearby conspecific (Karban and Baldwin 1997, Tollrian and Harvell 1999). Induction can increase an organism's fitness when defenses are costly (Baldwin 1998, Strauss et al. 2002), and may slow the counter-adaptation of natural enemies by creating variance in defenses that represent a moving evolutionary target (Karban et al. 1997, Agrawal 2011a, Karban 2011). As such, induction of chemical defense in response to herbivore attack has been widely documented among terrestrial plants (Karban and Baldwin 1997, Agrawal 2011a, Karban 2011), and may be common for marine and freshwater primary producers (Toth and Pavia 2007, Van Donk et al. 2011). In contrast induced chemical responses to competitors are less well studied in plants and macroalgae, and potential trade-offs between chemical traits to deter competitors vs. herbivores remain unclear (Strauss and Irwin 2004, Lankau and Strauss 2008, Inderjit et al. 2011, Paul et al. 2011a).

Numerous theories provide evolutionary reasoning for the differential production of anti-herbivore defenses within and among primary producers, and posit that the production of inducible anti-herbivore defenses may create a trade-off in allocation of resources to defense vs. growth, maintenance, or reproduction (Stamp 2003, Agrawal 2007, Agrawal 2011a, Karban 2011). However most of these theories were formed in the context of terrestrial plant-insect interactions and without regard to other processes (*e.g.,* competition, disease) that could affect the production of plant secondary metabolites.

These theories may thus be of limited utility in complex natural communities where primary producers such as macroalgae face a diversity of natural enemies and must use secondary chemistry to simultaneously mediate interactions with herbivores, competitors, and pathogens (Schmitt et al. 1995, Dworjanyn et al. 2006a, Hay 2009). True costs and trade-offs associated with chemical defenses are likely dependent on whether secondary metabolites serve single vs. multiple ecological functions, and how multiple ecological pressures simultaneously affect the productions of defense metabolites. Investigations addressing such complexities are limited for terrestrial plants (*e.g.*, Siemens et al. 2003, Strauss and Irwin 2004, Lankau and Strauss 2008, Agrawal 2011b, Kempel et al. 2011) and are largely absent for marine or aquatic macrophytes.

Trade-offs between chemical defense and basic metabolic functions like growth occur within macroalgae (Pavia et al. 1999), but trade-offs between the productions of secondary metabolites to deter competitors (allelochemicals) vs. herbivores (feeding deterrents) are poorly understood (Cipollini 2004). To reduce trade-offs and consolidate costs of defense, macroalgae can produce single compounds with both anti-herbivore and allelochemical functions, but this strategy may result in the adaptability of multi-function compounds being constrained by individual selective agents (Schmitt et al. 1995). Macroalgae producing multiple defensive compounds, each with separate ecological functions, may therefore be advantaged in nature due to the increased adaptability of their secondary chemistry to multiple ecological pressures that vary in time, space, and identity (Koricheva et al. 2004, Leimu and Koricheva 2006). However, whether macroalgae (1) commonly produce different secondary metabolites for differing ecological functions, (2)

experience trade-offs between the productions of such molecules, and (3) differentially express defensive molecules in response to competition vs. herbivory remains unclear.

Many tropical reefs have recently experienced dramatic losses of coral and increases in macroalgae due to a host of human disturbances (Mumby and Steneck 2008, Hughes et al. 2010). Phase-shifts from coral to macroalgal dominance result in increased algal-coral competition, and numerous macroalgae produce allelochemicals that reduce coral fitness (Rasher and Hay 2010, Paul et al. 2011b, Rasher et al. 2011) - forming ecological feedbacks that further limit coral recovery and promote macroalgal dominance (Mumby and Steneck 2008, Hughes et al. 2010). Thus, the plasticity of chemical defenses in macroalgae may be influenced not by just herbivory (Toth and Pavia 2007), but by multiple ecological processes (Strauss and Irwin 2004). If chemically rich macroalgae constitutively produce allelopathic compounds that also deter herbivores, or induce allelopathic metabolites following algal-coral contact that also have anti-herbivore activity, then algal-coral competition may further reduce herbivore control of macroalgae on degraded reefs and accelerate the ecological feedbacks that limit reef resilience. In contrast if algae produce different metabolites for deterring herbivores vs. coral competitors, and these metabolites involve reciprocal trade-offs, then macroalgae may become more susceptible to top-down control as a result of increased algal-coral competition on degraded reefs – potentially weakening positive feedbacks and enhancing the possibility for coral recovery. Investigations of how secondary chemistry in macroalgae link the processes of competition and herbivory on coral reefs would improve our understanding of the complexities of coral reef resilience, but are unexplored.

Here we assessed the effects of algal-coral competition on the induction of allelopathic vs. anti-herbivore chemical defenses in two tropical macroalgae that possess unique taxonomies, morphologies, and chemical profiles. In doing so, we asked: (1) Does algal-coral competition alter the production of allelochemicals or anti-herbivore chemical defenses in tropical macroalgae? (2) Do macroalgae produce different suites of chemical defenses for allelopathy vs. herbivore deterrence? (3) If so, is there a trade-off between the production of allelochemicals and anti-herbivore chemical defenses in macroalgae? (4) Do macroalgae vary by species in the above patterns and traits?

Materials and Methods

Experimental design, study site, and study organisms

Macroalgal induction experiments were conducted May-December 2011 on a 1-2 m deep reef flat within a no-take marine reserve adjacent to Votua village, Viti Levu, Fiji (18º13.049'S, 177º42.968'E). Within the reserve, reef-building corals are common (~57% cover) and macroalgae are rare (~3% cover). Adjacent areas subject to artisanal fishing are dominated by macroalgae (~47% cover) and corals are rare (~3% cover) (Rasher and Hay 2010). We used the brown alga *Sargassum polycystum* and the red alga *Galaxaura filamentosa* in our study (hereafter referred to by genus names) because they (1) are common on the non-reserve reefs, (2) are regularly observed in contact with corals in degraded habitats, (3) represent divergent taxonomic and morphological forms, and (4) vary in their palatability to consumers (*Sargassum* = high, *Galaxaura* = low) and allelopathic effects on coral (*Sargassum* = low, *Galaxaura* = high) (Rasher and Hay 2010, Rasher et al. 2011). We used the coral *Porites cylindrica* (hereafter "*Porites*") in our study because it (1) is an abundant coral on reef flats in Fiji and (2) responds

differentially to competition with differing algae, but (3) is relatively resistant to the allelopathic effects of macroalgae compared to other co-occurring corals (Rasher and Hay 2010, Rasher et al. 2011).

Induction of macroalgal allelochemicals

We fragmented small branches of *Porites* (6-8 cm in length) from within the reserve, epoxied fragments into cement cones, and deployed these on a metal rack at a depth of 1 m in the marine reserve for 24 months to allow acclimation and growth. This produced 100 multi-branched fragments approximately 15 cm in height. Immediately prior to induction experiments, half of the corals (n = 50) were removed, submerged in 100% bleach for 4 d, rinsed and dried to remove all traces of bleach, and interspersed among the 50 living corals on the rack to serve as induction controls (*i.e.*, to control for algal chemical induction due to abrasion and shading from an adjacent hard substrate).

To manipulate algal-coral competition, we collected 25 large individuals of both *Sargassum* and *Galaxaura* from the non-reserve, and (for each species) split each alga into two similar sized portions to standardize genotype-specific variance in chemical plasticity and initial levels of chemical defenses between treatments. We then uniformly spun each pair in a salad spinner to remove excess water, weighed each, inserted each into a 20 cm segment of three-stranded rope, and attached one thallus of each pair to either a cone holding a living *Porites* ("live-contact") or a cone holding a *Porites* skeleton ("skeleton-contact") (n = 25 treatment^{-1} species^{-1}). To prevent herbivores from confounding our treatments, we caged the experimental rack with wire mesh (1 cm^2 grid). After 8 d of contact, we collected, spun, and reweighed the algae (as above) to determine growth differences between treatments. Some thalli of each species detached during the

8 d study; these replicates and their autogenic pairs were excluded, resulting in n = 15 treatement^{-1} species^{-1}.

To assess allelochemical induction in *Sargassum* and *Galaxaura* after 8 d of coral contact, we generated hydrophobic extracts from live- and skeleton-contact thalli and tested their allelopathic activity against fresh *Porites* fragments in the field. For each algal species, a small portion of each thallus was removed, grouped by treatment, and extracted in 100% methanol. Extracts were then each dried by rotary evaporation, partitioned between water and ethyl acetate, and the hydrophobic (ethyl acetate) fraction of each was retained for testing its effects on *Porites*. Extracts from the live-contact and skeletal-contact treatments were re-suspended in methanol and each incorporated at natural concentration into a series of 1 cm^2 Phytagel squares (n = 10 extract^{-1} species^{-1}) hardened on window screen backing (Thacker et al. 1998, Rasher and Hay 2010, Rasher et al. 2011). Control gels contained solvent, but lacked algal extract (n = 10). Treatment and control Phytagel squares were wrapped and cable-tied at mid-height on individual fragments of *Porites* (6-8 cm in height, planted as above) and interspersed on an un-caged rack in the reserve. After 24 h, we removed each square and assessed the effects of extracts vs. controls on coral photophysiology by taking a single pulse amplitude modulated (PAM) fluorometry measurement (fiberoptic diameter = 5.5 mm, distance = 9-10 mm, angle = perpendicular) under the center of each square.

To confirm that allelochemical induction by *Galaxaura* actually increases coral damage, we conducted an 8 d *Galaxaura–Porites* contact manipulation identical to that described above, but after 8 d we placed whole thalli of live- and skeleton-contact *Galaxaura* in contact with fresh living *Porites* fragments (6-8 cm in height) in cones (n =

15 treatment^{-1}) and assessed the differential effects of the algal treatments on coral photophysiology over 12 d. We deployed *Porites* not in contact with macroalgae as environmental controls (n = 15). On days 2, 4, and 12 we took a single PAM fluorometry measurement (as above) on each treatment coral at the most damaged location experiencing algal contact along the mid-point of the fragment (*i.e.*, excluding coral extremities). Controls were sampled (using identical protocol) at the most damaged location at approximately the same height as treatments. We caged the rack to exclude large herbivores.

PAM fluorometry

PAM fluorometry was used to assess PSII quantum yield of the symbiotic microalgae (zooxanthellae) living within *Porites*. PAM fluorometry is used to investigate physiological responses of the coral holobiont to biotic or abiotic stressors, and the processes leading to coral bleaching (Warner et al. 1999, Fitt et al. 2001, Smith et al. 2006, Pawlik et al. 2007, Rasher and Hay 2010, Rasher et al. 2011). Measurements of light-adapted corals [*i.e.*, effective quantum yield (Φ_{PSII})] theoretically range from 0.0 to ~0.83. Empirical studies suggest that values in the range of ~0.5-0.75 are indicative of a healthy coral and values of ~0.0-0.25 are indicative of bleaching and mortality (Warner et al. 1999, Fitt et al. 2001, Smith et al. 2006, Pawlik et al. 2007, Rasher and Hay 2010, Rasher et al. 2011). Although PAM fluorometry does not quantify coral bleaching *per se*, PAM fluorometry measurements are highly correlated with visual assessments of bleaching for *Porites* and other co-occurring corals at our study site (Rasher and Hay 2010, Rasher et al. 2011).

We sampled corals between 0900-1300 h. Readings for treatments and controls were interspersed through time to prevent confounding treatment effects with *in situ* diurnal changes in non-photochemical quenching (*i.e.*, temperature and UV). Coral fragments were (1) collected from colonies adjacent to our experimental rack (*i.e.*, from the same depth and local condition), (2) allowed to acclimate on the rack for at least one month prior to experiments, and (3) interspersed among treatments and controls to minimize and homogenize any initial variance in zooxanthellae density and diversity among replicates. Care was taken to avoid self-shading during *in situ* measurements.

Macroalgal palatability and anti-herbivore defense

Following 8 d of contact with *Porites*, we removed four branches from each live-contact alga, spun them uniformly in a salad spinner to remove excess water, weighed the branches, and inserted them 5 cm apart on a 60 cm section of three-stranded rope (n = 15 ropes species^{-1}). Ropes holding autogenic pairs of skeleton-contact algae were assembled in the same manner. A single branch of each alga from both treatments was also spun, weighed, and inserted into a 20 cm segment of three-stranded rope to be deployed as a caged control to assess algal tissue loss during assays that was unrelated to herbivory.

Within the reserve, we deployed autogenic pairs of live- and skeleton-contact algae (n = 15 pairs species^{-1}) in a network of pools accessible to herbivores at low (~1m depth) and high (~2m depth) tides. We deployed paired ropes within ~0.5-0.75 m of each other, deployed autogenic caged controls within 1 m of each pair, and separated replicate pairs by 5-7 m. We recollected pairs when ~50% of the total biomass was consumed; thus feedings assays of *Sargassum* lasted 2-24 h and assays of *Galaxaura* lasted 5-7 d. Post-assay algae were carefully bagged *in situ*, returned to the laboratory, spun, and re-

weighed. The mass of each alga consumed was calculated using the formula [T_i x (C_f / C_i)] - T_f, where T_i and T_f were the initial and final masses (respectively) of a treatment alga exposed to herbivory, and C_i and C_f were the initial and final masses (respectively) of its autogenic caged control excluded from herbivory. Percentage of each alga consumed was calculated to facilitate comparisons.

To elucidate whether an increase in feeding on *Galaxaura* following 8 d of contact with live *Porites* was due to a relaxation of anti-herbivore defensive chemistry, we tested the deterrent effects of hydrophobic extracts from live- vs. skeleton-contact *Galaxaura* on herbivorous fishes in the field. To assay extracts against herbivorous surgeonfishes (*Naso lituratus* and *Naso unicornis*) we re-suspended each extract in ether, coated a natural volumetric concentration of this extract on five blades [a 2.04 ± 0.03 mL volumetric equivalent (mean ± SE); n = 10] of blotted and pre-weighed *Padina boryana* [a prey of *N. lituratus* and *N. unicornis* (Meyer et al. 1994, Choat et al. 2002, Chapter 4); hereafter "*Padina*"], allowed the ether to evaporate, and inserted these blades each 5 cm apart on a 60 cm section of three-stranded rope (n = 12 ropes extract^{-1}).

Ropes holding *Padina* coated with extracts of live-contact *Galaxaura* were paired with ropes holding *Padina* coated with extracts of skeleton-contact *Galaxaura*. Paired ropes were placed within 0.5-0.75 m of each other and replicate pairs were separated by 5-7 m in the field. Because feeding on *Padina* was rapid (~10 min pair^{-1}), we deployed one pair at a time, monitored feeding, and recollected pairs when ~50% of the total algal mass was consumed. Only *Naso lituratus* and *N. unicornis* were observed feeding on *Padina* in these assays. Post-assay ropes were carefully bagged in the field, and blotted and re-weighed in the laboratory. Given the short-term nature of each assay and our

visual assessment that algal portions were not being lost to processes other than herbivory, we did not deploy caged controls.

To assay extracts of *Galaxaura* against other herbivores such as parrotfishes, we re-suspended the same algal extracts in ether, coated a natural volumetric concentration of each extract on three branches (a 0.74 ± 0.02 mL volumetric equivalent) of blotted and pre-weighed *Amphiroa crassa* [a heavily calcified alga likely avoided by *Naso* and consumed by parrotfishes (Schupp and Paul 1994, Chapter 4)], allowed the ether to evaporate, and inserted these branches each 5 cm apart on a 60 cm section of three-stranded rope (n = 12 ropes extract^{-1}). *Amphiroa crassa* (hereafter "*Amphiroa*") coated only with ether were also deployed in cages within 1 m of each pair to control for changes in algal mass unrelated to grazing. Field feeding assays were conducted as above. Grazing rates on *Amphiroa* were low (only 5 pairs received detectable grazing) and assays lasted 3-5 d. Consumption of *Amphiroa* among the 5 pairs with grazing was calculated using the formula described above.

Ecological functions and trade-offs of secondary chemistry

To evaluate if different *Galaxaura* metabolites deter herbivores vs. competitors, we extracted *Galaxaura* collected from the reef, partitioned the extract into fractions based on molecular polarity, and tested the fractions for their bioactivity against herbivores vs. the coral *Porites*. Initial extraction was in 100% methanol. We then partitioned the crude extract between hexanes (fraction A) and 9:1 methanol/water, altered the 9:1 methanol/water fraction to 6:4 methanol/water and partitioned it against chloroform (fraction B), and finally altered the 6:4 methanol/water fraction to 100%

water (fraction D) and partitioned it against ethyl acetate (fraction C). Fractions A-D were each dried by rotary evaporation and stored at -5^{o}C until used in bioassays.

For allelopathy assays, we embedded each fraction at natural volumetric concentration into Phytagel squares (n = 10 extract^{-1}) and tested the allelopathic effects of each fraction (relative to control gels) on *Porites* as described above. To test anti-herbivore deterrence, we coated each fraction on *Padina* at natural volumetric concentration, paired these individuals with control *Padina* coated only with solvent, and deployed pairs (n = 10 fraction^{-1}) in the field as described above. We tested one fraction per day. On two days, no grazing occurred. Given time constraints in the field, we were forced to combine fractions A and B for a single assay of chemical herbivore deterrence for these fractions. Fractions C and D were tested individually.

Statistical analysis

Because live- and skeleton-contact treatments were created using autogenic pairs of algae, we evaluated data for algal growth, palatability, and extract palatability with paired t tests (and in one instance a Wilcoxon signed rank test because the data could not be transformed to meet parametric assumptions). Extract allelopathy data were evaluated with a one factor Analysis of Variance (ANOVA). We evaluated data from our allelochemical induction experiment using whole *Galaxaura* thalli with a two factor, repeated measures ANOVA. We evaluated bioactivity data of *Galaxaura* extract fractions as allelochemicals and anti-herbivore defenses using a one factor ANOVA and paired t tests, respectively. Significant ANOVA results were further evaluated with Tukey HSD post-hoc tests.

Results

Induction of allelochemicals in macroalgae

Hydrophobic extracts of *Galaxaura filamentosa* and *Sargassum polycystum* thalli (hereafter referred to by their genus names) were created following 8 d of contact with the coral *Porites cylindrica* ("live-contact") or only skeletons of *P. cylindrica* ("skeleton-contact"). When embedded in Phytagel squares at natural concentration and applied to *P. cylindrica* (hereafter "*Porites"*) for 24 h in the field, extracts of both live- and skeleton-contact *Galaxaura* significantly reduced the quantum yield (Φ_{PSII}) of zooxanthellae within *Porites* relative to controls (ANOVA: $F_{4, 45} = 36.676$, $p < 0.001$; Figure 5.1A), but extracts of live-contact *Galaxaura* caused a 44% greater reduction of Φ_{PSII} than did skeleton-contact extracts (see post-hoc results, Figure 5.1A). This pattern suggests an induction of allelopathic compounds in *Galaxaura* in response to contact with a living coral. As a result of competition, growth of live-contact *Galaxaura* was suppressed by 73% when compared to growth of skeleton-contact thalli (3.09 ± 1.96% (mean ± SE) vs. 11.38 ± 3.79%; paired t test: $p = 0.044$). In contrast to *Galaxaura*, hydrophobic extracts of live- and skeleton-contact *Sargassum* did not alter Φ_{PSII} of zooxanthellae within *Porites* relative to controls (see post-hoc results, Figure 5.1A), and growth of *Sargassum* did not differ between live-contact (12.42 ± 3.78%) and skeleton-contact (13.13 ± 5.11%) thalli (paired t test: $p = 0.910$).

When *Galaxaura* thalli were placed in contact with new *Porites* following 8 d of previous contact with *Porites* or its skeleton, live-contact thalli initially (day 2) caused a significantly greater reduction of zooxanthellae Φ_{PSII} within *Porites* than did skeleton-

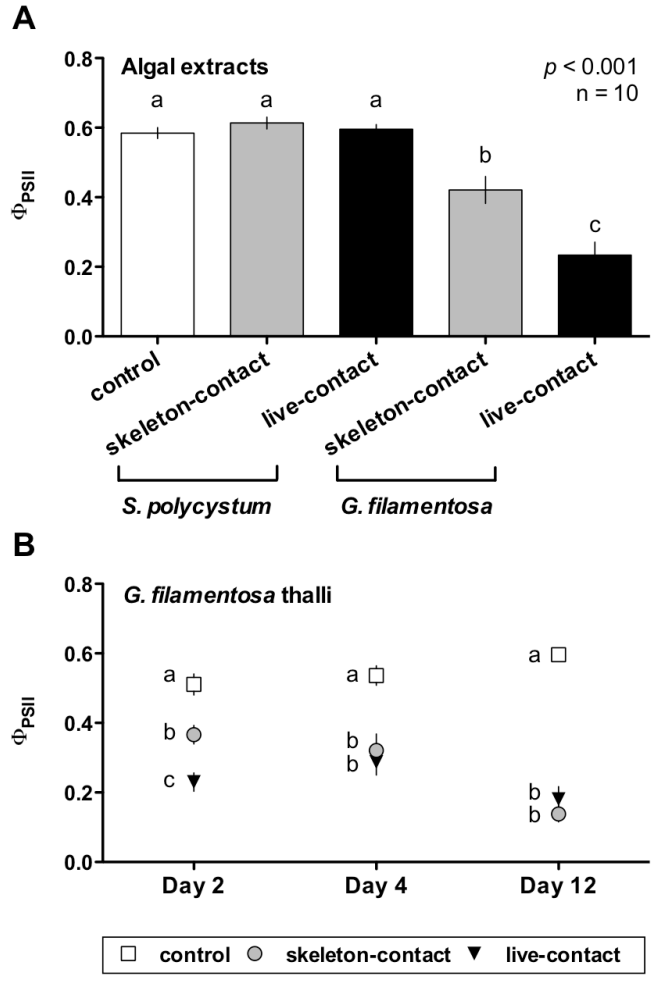

Figure 5.1: Algal induction of allelopathy in response to coral contact. (A) PSII quantum yield (Φ_{PSII}; mean ± SE) of zooxanthellae within the coral *Porites cylindrica* after 24 h of contact with Phytagel squares containing hydrophobic extracts of *Galaxaura filamentosa* or *Sargassum polycystum* thalli that had been in contact with living *Porites* ("live-contact"; n = 10) vs. in contact with *Porites* skeletons for 8 d ("skeleton-contact"; n = 10), relative to solvent-only Phytagel controls (n = 10). (B) PSII quantum yield (Φ_{PSII}; mean ± SE) of zooxanthellae within *Porites* after 2, 4, and 12 d of contact with live-contact thalli (n = 15) or skeleton-contact thalli (n = 15) of *Galaxaura*, compared to *Porites* lacking algal contact ("control"; n = 15). Evaluated by a one factor ANOVA (A) or a two factor, repeated measures ANOVA (B). Letters indicate significant groupings among treatments (A) or among treatments within days (B) by Tukey HSD post-hoc tests. Complete ANOVA and additional Tukey HSD post-hoc test results for (B) are in Table 5.1.

contact thalli (Figure 5.1B; Table 5.1). This initial difference in allelopathic activity diminished rapidly; by day 4, both live- and skeleton-contact thalli significantly reduced Φ_{PSII} to a similar degree (Figure 5.1B), indicating that induction of greater allelopathy by *Galaxaura* occurs rapidly (within 2-4 d) following initial contact with coral. Allelopathic effects of *Galaxaura* on corals increased in magnitude for both treatments from days 4 to 12 (Table 5.1), resulting in a similar and significant reduction of Φ_{PSII} after 12 d relative to control corals (Figure 5.1B). Effects of *Galaxaura* on corals did not differ between days 2 and 12 for live-contact thalli (which showed strong effects throughout the 12 d experiment), but effects of skeleton-contact thalli on corals significantly increased with time – consistent with induction of allelochemicals in these algae in response to new contact with living coral (Table 5.1). Quantum yield of zooxanthellae within control corals did not change significantly during the study (Table 5.1).

Table 5.1: Complete two factor, repeated measures ANOVA results and additional Tukey HSD post-hoc test results for data presented in Figure 5.1B. Significant *p* values are in bold.

Source of Variation	*df*	SS	MS	*F*	*p*
Treatment	2	2.629	1.314	73.123	**<0.001**
Replicate (Treatment)	42	0.755	0.018		
Day	2	0.152	0.076	5.952	**0.004**
Treatment x Day	4	0.429	0.107	8.407	**<0.001**
Residual	84	1.072	0.013		
Total	134	5.037	0.038		

Tukey HSD	**Treatment**		
	live-contact	skeleton-contact	control
Day 2 vs. 4	0.346	0.517	0.807
Day 2 vs. 12	0.462	**<0.001**	0.102
Day 4 vs. 12	**0.030**	**<0.001**	0.322

Macroalgal palatability and anti-herbivore chemical defense

The palatability of *Sargassum* to herbivores in the field did not differ between live-contact and skeleton-contact thalli (paired t test: $p = 0.632$; Figure 5.2A). In contrast for *Galaxaura*, herbivores consumed 84% more of live-contact thalli than skeleton-contact thalli (paired t test: $p = 0.025$; Figure 5.2B), suggesting a relaxation of anti-herbivore defenses or an increase in nutritional quality of *Galaxaura* in response to algal-coral competition.

Consistent with a relaxation of anti-herbivore chemical defenses in *Galaxaura* following 8 d of contact with *Porites*, herbivores consumed 2.4 times more *Padina boryana* coated with hydrophobic extracts of live-contact *Galaxaura* than *Padina* coated with extracts of skeleton-contact *Galaxaura* (Wilcoxon signed rank test: $p = 0.021$; Figure 5.3A). Similarly, herbivores consumed 3.8 times more of *Amphiroa crassa* coated with hydrophobic extracts of live-contact *Galaxaura* than *Amphiroa* coated with extracts of skeleton-contact *Galaxaura* (paired t test: $p = 0.020$; Figure 5.3B).

Ecological functions and trade-offs of secondary chemistry

When a chemical extract of *Galaxaura* was divided by polarity into four fractions and each fraction was incorporated into Phytagel squares at natural concentration and applied onto *Porites* for 24 h in the field, only fraction C reduced Φ_{PSII} of zooxanthellae within *Porites* relative to controls (ANOVA: $F_{4, 45} = 4.492$, $p = 0.004$; Figure 5.4). In contrast when these same fractions were each coated onto the palatable alga *Padina boryana* at natural concentration and exposed to herbivores in the field, fraction C had no deterrent effect (paired t test: $p = 0.177$; Figure 5.5). Only fraction A + B reduced fish

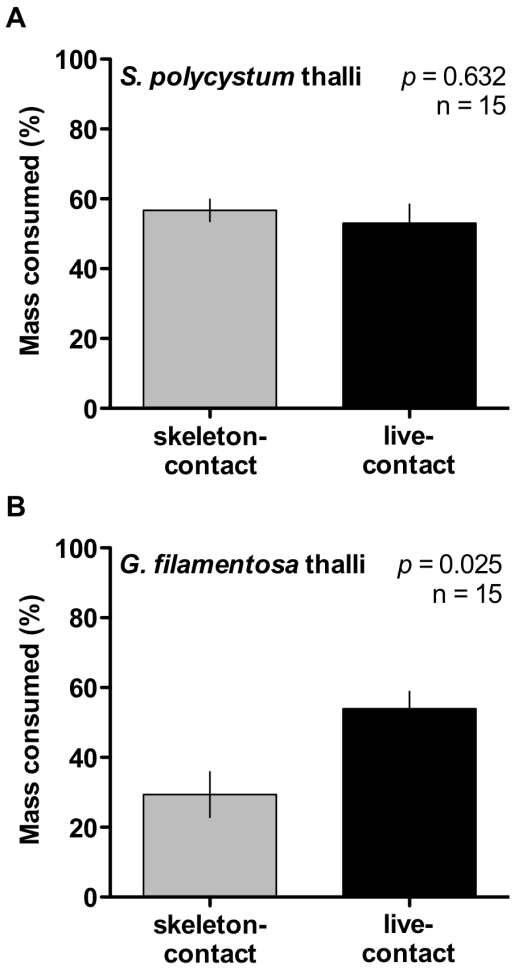

Figure 5.2: Changes in algal palatability due to coral contact. Mass (mean ± SE) of (A) *Sargassum polycystum* or (B) *Galaxaura filamentosa* consumed by herbivores in the field, for thalli previously in contact with the coral *Porites cylindrica* for 8 d (live-contact) vs. paired thalli previously in contact with skeletons of *Porites* (skeleton-contact) for 8 d (n = 15 pairs species^{-1}). Evaluated by paired t tests.

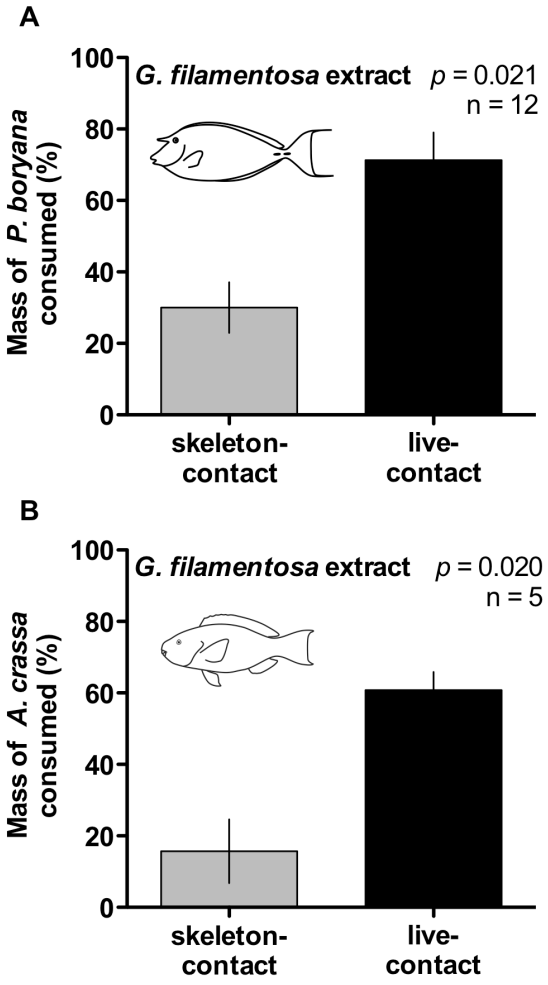

Figure 5.3: Changes in algal chemical defense against herbivores due to coral contact. Mass (mean ± SE) of (A) *Padina boryana* consumed by the surgeonfishes *Naso lituratus* and *Naso unicornis* (n = 12) or (B) *Amphiroa crassa* likely consumed by parrotfishes (n = 5) in the field, for thalli coated with hydrophobic extracts of live-contact *Galaxaura filamentosa* vs. paired thalli coated with extracts of skeleton-contact *Galaxaura*. Evaluated by a Wilcoxon signed rank test (A) or a paired t test (B).

grazing relative to paired controls (paired t test: $p < 0.001$; Figure 5.5). We were unable to separately test fractions A and B due to constrained field time and conditions, but one or both clearly deter herbivory and neither is allelopathic.

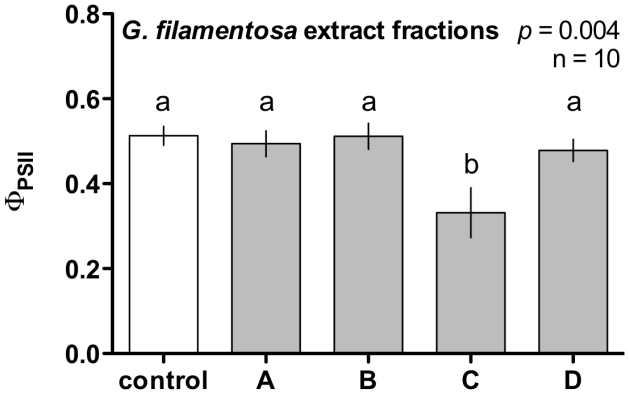

Figure 5.4: PSII quantum yield (Φ_{PSII}; mean ± SE) of zooxanthellae within *Porites cylindrica* after 24 h of contact with Phytagel squares containing one of four extract fractions from *Galaxaura filamentosa* (n = 10 fraction^{-1}) or solvent only ("control"; n = 10). Fractions A-D are arranged from least to most polar. Evaluated by a one factor ANOVA. Letters indicate significant groupings by a Tukey HSD post-hoc test.

Discussion

Secondary chemistry plays a key role in mediating pair-wise interactions between macroalgae and herbivores, competitors, and fouling or disease agents (Engel et al. 2002, Toth and Pavia 2007, Hay 2009, Paul et al. 2011a, Rasher et al. 2011), as also occurs in other systems (Legrand et al. 2003, Inderjit et al. 2011, Karban 2011, Sieg et al. 2011, Van Donk et al. 2011). Yet organisms do not interact as species pairs; they interact with a complex web of other species, simultaneously confronting a diversity of consumers, competitors, and pathogens (Strauss and Irwin 2004). Theoretical and empirical

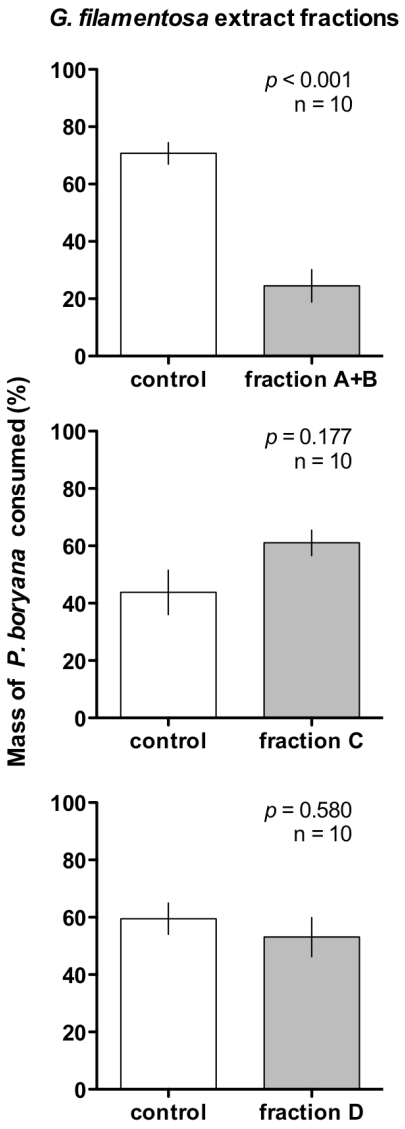

Figure 5.5: Mass (mean ± SE) of *Padina boryana* consumed by herbivores in the field, for thalli coated with the same *G. filamentosa* chemical fractions as shown in Figure 5.4 vs. paired thalli coated with solvent only (n = 10 pairs extract^{-1}). Evaluated by paired t tests.

investigations of how differing ecological processes affect the induction of secondary metabolites with individual vs. multiple ecological roles are rare for aquatic producers, when compared to terrestrial plants (Siemens et al. 2002, Siemens et al. 2003, Biere et al. 2004, Thelen et al. 2005, Lankau and Strauss 2008, Lankau and Kliebenstein 2009). On a coral reef we demonstrate that in response to competition with the coral *Porites cylindrica*, the chemically rich macroalga *Galaxaura filamentosa* simultaneously induces allelochemicals against competitors (Figure 5.1; Table 5.1) and decreases chemical defenses against herbivores (Figures 5.2 and 5.3) - likely due to a trade-off in the production of differing metabolites responsible for deterring herbivores vs. competitors (Figures 5.4 and 5.5). *Sargassum polycystum* is not allelopathic to coral (Rasher and Hay 2010, Rasher et al. 2011) and did not respond chemically to algal-coral competition (Figures 5.1-5.3). Thus, chemical responses of marine macroalgae to competitors and herbivores are not uniform between species, and may be more nuanced and complex than is generally appreciated.

Numerous theories focused on the evolution of plant chemical defense against herbivores assume that chemical defenses are costly and that allocation to defense constrains basic metabolic function, yet costs are rarely considered in a community context and allocations to one defense are rarely considered in terms of constraints on other defenses (Strauss et al. 2002, Strauss and Irwin 2004, Dworjanyn et al. 2006b, Auld et al. 2010). The reduced growth of *Galaxaura* and its increased palatability to herbivores following an induction of allelochemicals when competing with a coral suggests that *Galaxaura* incurs an 'ecological cost' (*sensu* Strauss et al. 2002) when inducing allelopathy. In contrast *Sargassum* did not induce allelochemicals, experience

reduced growth, or experience a change in palatability following contact with *Porites* – all indicating a lack of chemically mediated trade-offs, or chemical defenses in general, for this non-allelopathic species.

Trade-offs between defenses against competitors and herbivores can occur in terrestrial plants due to a conflict in allocation of limited resources to differing defenses, or due to interference between hormonal pathways regulating the productions of metabolites with differing ecological functions (Cipollini 2004). Our marine study detected a similar constraint, where inducing greater allelopathy resulted in compromised chemical defense against herbivores (Figures 5.1-5.3). However, our findings contrast with other studies of a marine macroalga (Schmitt et al. 1995), marine sponges (Kubanek et al. 2002), and terrestrial plants (*e.g.*, Biere et al. 2004), which found that the same chemical defenses were effective against both competitors/disease agents and predators. When secondary metabolites fulfill multiple ecological roles, trade-offs and overall costs of defense may be reduced or obscured. For *Galaxaura*, chemical defenses against herbivores and a common competitor involve different compounds (Figures 5.4 and 5.5), and inducing allelopathy constrains chemical defense against herbivores.

Coral reef resilience is critically dependent on the process of herbivory (Hughes et al. 2007, Burkepile and Hay 2008, Adam et al. 2011), which prevents the establishment of competitively superior algae and thus promotes coral recovery following disturbances (Hughes et al. 2010). Yet on many reefs experiencing coral decline, the loss of key herbivores to overfishing and habitat degradation has led to a proliferation of macroalgae that inhibit coral survivorship, growth, and recruitment thereby creating ecological feedbacks that promote macroalgal dominance (Mumby and Steneck 2008, Hughes et al.

2010). Chemically rich macroalgae that produce the same secondary metabolites to both deter herbivores (Hay et al. 1988, Schupp and Paul 1994) and reduce coral survival and recruitment (Rasher and Hay 2010, Paul et al. 2011b, Rasher et al. 2011) could pose an elevated threat to reef resilience by accelerating the ecological feedbacks limiting coral recovery on degraded reefs. This would constitute a "worst-case scenario."

We found that the chemically rich alga *Galaxaura* produces different compounds for deterring herbivores vs. suppressing a coral competitor (Figures 5.4 and 5.5), and that its induction of allelochemicals following coral contact (Figure 5.1) coincided with a reduction of anti-herbivore defense (Figures 5.2 and 5.3). If the chemically mediated trade-off we documented for *Galaxaura* is representative of allelopathic macroalgae in general, increases in algal-coral competition could result in increased herbivore control of allelopathic algae and would counter the worst-case scenario mentioned above.

Secondary chemistry plays an important role in governing many processes that affect marine populations, communities, and ecosystems (Hay 2009). However, our understanding of how algal secondary chemistry simultaneously affects multiple ecological processes is in its infancy. This study demonstrates that algal-coral competition simultaneously induces allelopathic metabolites and decreases anti-herbivore chemical defenses in a macroalga – a pattern likely due to a trade-off in the production of molecules with differing ecological functions. Further, this phenomenon was species-specific. Such findings highlight the need to incorporate greater ecological complexity into theoretical and empirical investigations aimed at explaining the ecology and evolution of chemical defenses (Siemens et al. 2003, Strauss and Irwin 2004, Lankau and

Strauss 2008), as well as the cascading effects of these defenses on communities and ecosystems (Hay 2009).

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APPENDIX A

SUPPORTING INFORMATION FOR CHAPTER 2

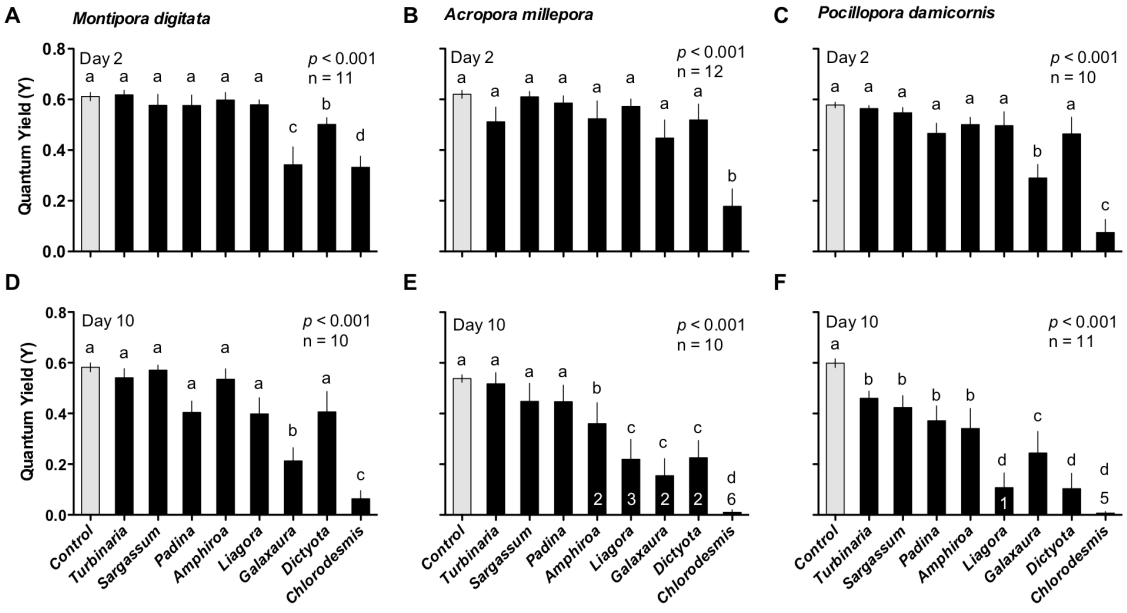

Figure A.1: Effects of macroalgae on corals on days 2 and 10. Effective quantum yield (Y; mean ± SE) of the corals (A & D) *Montipora digitata* (n = 11), (B & E) *Acropora millepora* (n = 12), and (C & F) *Pocillopora damicornis* (n = 10) when in direct contact with macroalgae for 2 d (A-C) and 10 d (D-F), relative to controls (n = 10-12). Analyzed by Kruskal-Wallis Analysis of Variance on ranks. Letters indicate significant groupings by post-hoc Student-Newman-Kuels tests. Numbers inset within bars indicate number of replicates experiencing 100% mortality.

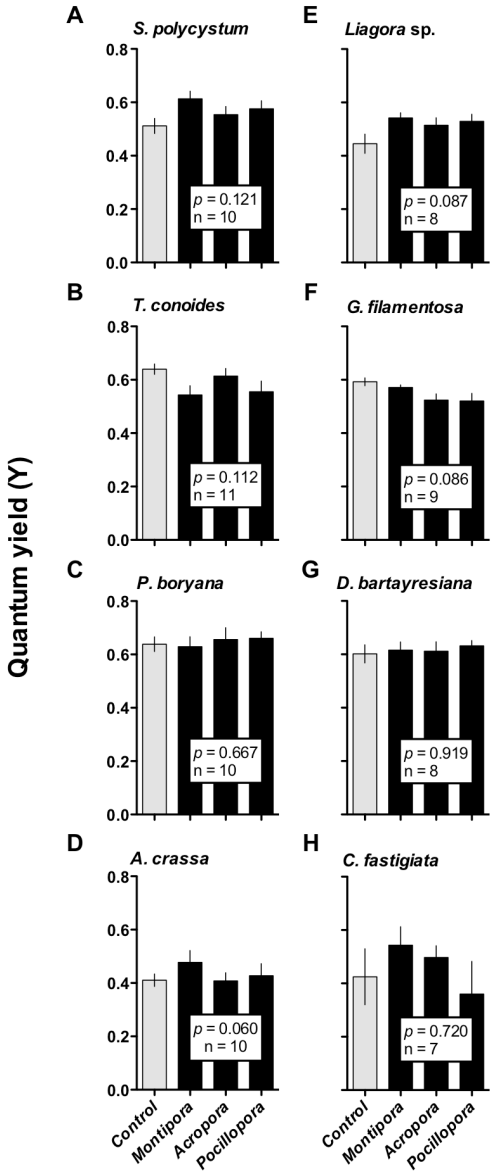

Figure A.2: Effects of corals on macroalgae. Effective quantum yield (Y; mean ± SE) of common macroalgae when in contact with the corals *Montipora digitata*, *Acropora millepora*, or *Pocillopora damicornis*, relative to control algae lacking contact with coral (n = 7-11). Analyzed by one factor Analysis of Variance (ANOVA), or by Kruskal-Wallis ANOVA on ranks if parametric assumptions were violated.

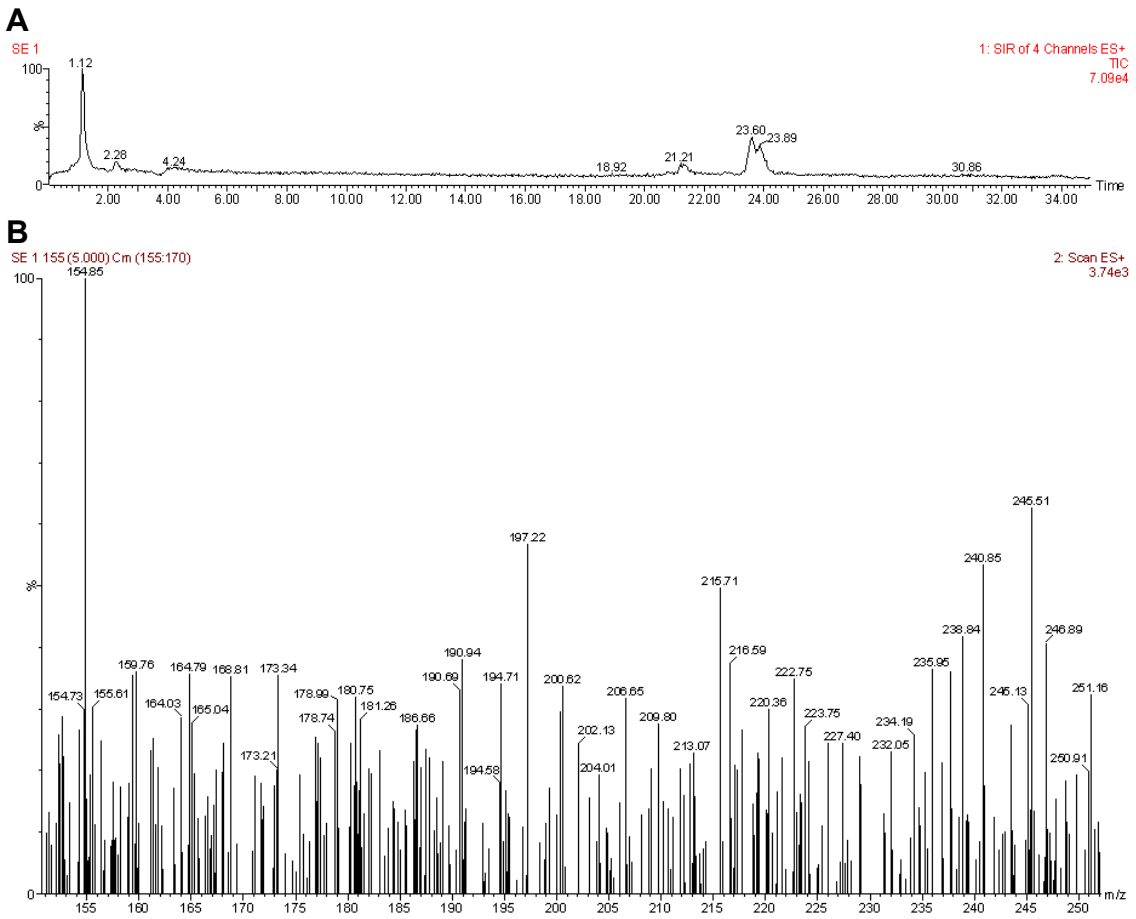

Figure A.3: Representative LC-MS chromatogram of *Galaxaura filamentosa* surface extracts. Retention times of allelochemicals **1** and **2** (Figure 2.5) in surface extracts were consistent with pure compounds isolated from whole cell extracts. (A) Selected ion recording (SIR) of [M+H]+ and [M+Na] for both compounds **1** and **2**, eluting between 4.5-5.0 minutes. (B) Mass spectrum representing the presence of allelochemicals **1** and **2**; [M+H]+ = 215.71 for allelochemical **1** and 197.22 for allelochemical **2**.

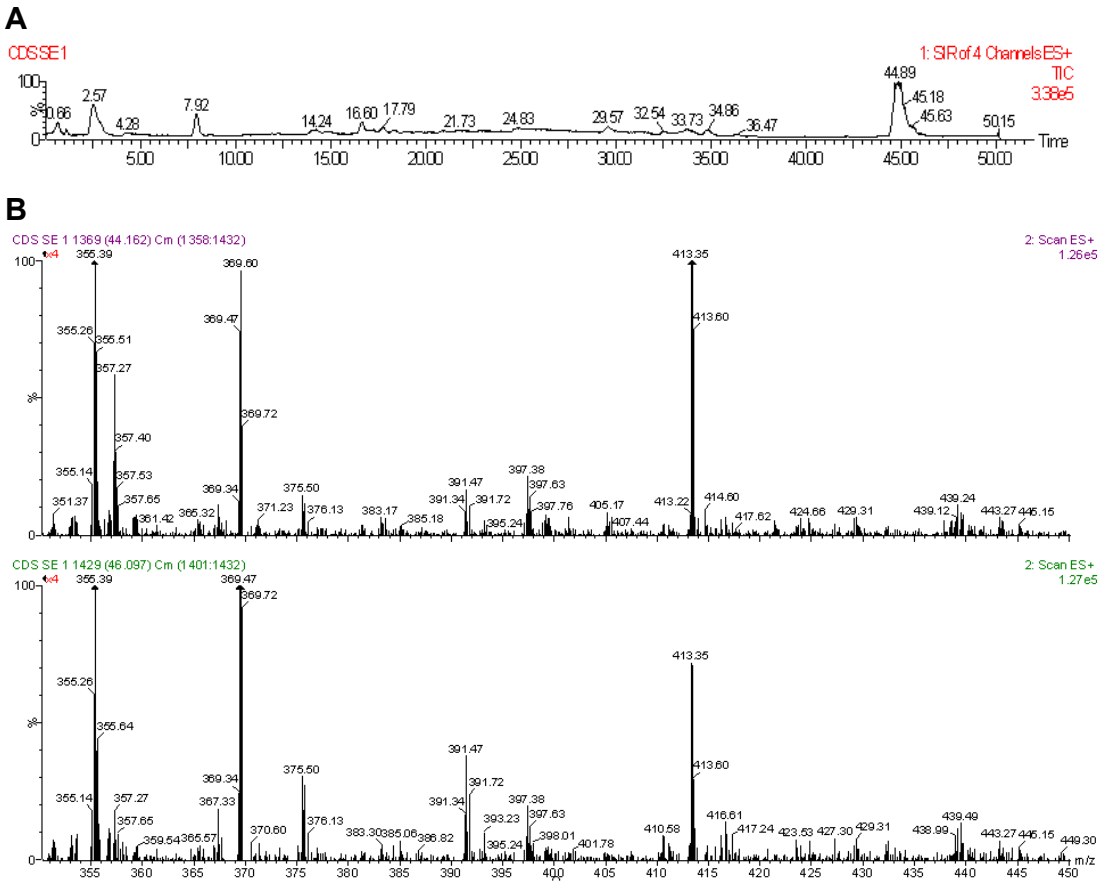

Figure A.4: Representative LC-MS chromatogram of *Chlorodesmis fastigiata* surface extracts. Retention times of allelochemicals **3** and **4** (Figure 2.5) in surface extracts were consistent with pure compounds isolated from whole cell extracts. (A) Selected ion recording (SIR) of [M+H]+ and [M+Na] for both compounds **3** and **4**, eluting between 44-46 minutes. (B) Mass spectra representing the presence of allelochemicals **3** and **4**; [M+Na] = 413.35 for allelochemical **3** (top) and [M+Na] = 417.24 for allelochemical **4** (bottom).

Table A.1: Cell lysis data for *Galaxaura filamentosa* and *Chlorodesmis fastigiata* following the extraction of surface-associated allelochemicals using hexanes for 30 seconds vs. a seawater control for 30 seconds.

Species	Extraction	Mean % cells lysed	SE	n	Mann-Whitney U test
G. filamentosa	seawater	2.41	0.67	5	$p = 0.421$
G. filamentosa	hexanes	1.51	0.31	5	$p = 0.421$
C. fastigiata	seawater	2.00	2.00	5	$p > 0.999$
C. fastigiata	hexanes	2.00	2.00	5	$p > 0.999$

Table A.2: ^{13}C and ^{1}H NMR spectroscopic data for compounds **1** and **2**, isolated from *Galaxaura filamentosa* ($CDCl_3$; 800 MHz).

	Compound **1**		Compound **2**	
Position #	δ_C*	δ_H	δ_C	δ_H
1	37.0	-	35	-
2	46.2	1.43 m, 1.83 ddd	49.5	1.32 m, 2.01ddd
3	63.8	3.89 m	64.6	4.11 m
4	47.7	1.51 m, 2.40 ddd	47	1.49 m, 2.52 ddd
5	88.2	-	86	-
6	81.8	-	180	-
7	41.3	2.37 d, 2.95 d	112.8	5.69 s
8	173.6	-	170	-
9	27.0	1.01 s	24.8	1.25 s
10	21.1	1.07 s	29.4	1.30 s
11	20.8	1.52 s	25.3	1.57 s

d = doublet; ddd = doublet of doublet of doublets; m = multiplet; s = singlet; t = triplet

*Carbon assignments were based on heteronuclear single-quantum correlation spectroscopy and heteronuclear multiple bond correlation data collected at 800 MHz.

Table A.3: ^{1}H NMR spectroscopic data for compounds **3** and **4**, isolated from *Chlorodesmis fastigiata* ($CDCl_3$; 500 MHz).

Position #	Compound **3** δ_H	Compound **4** δ_H
1	4.63 d	7.36 d
2	5.61 t	5.87 d
4	2.12 m	2.23 m
5	2.11 m	2.10 m
6	5.10 t	5.16 t
8	2.00 m	2.04 m
9	2.01 m	2.01 m
10	5.12 t	5.23 t
12	2.02 m	3.02 s
13	2.02 m	-
14	5.10 t	3.25 s
16	4.54 s	7.12 s
17	1.61 s	1.60 s
18	1.61 s	1.60 s
19	1.60 s	9.45 s
20	1.68 s	6.14 s, 6.29 s
OAc	2.11 s	2.13 s
	2.09 s	2.11 s

d = doublet; m = multiplet; s = singlet; t = triplet

APPENDIX B

SUPPORTING INFORMATION FOR CHAPTER 3

Table B.1: Results from three factor analysis of variance (ANOVA) of algal percent cover data. Data were rank-transformed. Significant results are highlighted in bold.

Effect	*df*	Total algal cover (%)		Upright macro-algae (%)		Crustose coralline algae (%)		Algal turf <0.5 cm (%)		Algal turf >0.5 cm (%)		Cyanobacteria (%)	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Herbivory (H)	1	175.522	**<0.001**	78.263	**<0.001**	42.384	**<0.001**	80.638	**<0.001**	1.076	0.303	15.059	**<0.001**
Nutrients (N)	1	0.014	0.906	0.095	0.759	0.544	0.463	0.067	0.797	0.975	0.327	2.473	0.120
Site (S)	1	1.372	0.245	0.204	0.653	2.315	0.133	2.007	0.161	0.001	0.980	13.649	**<0.001**
H × N	1	6.800	**0.011**	0.225	0.637	2.456	0.121	3.295	0.074	0.001	0.980	8.430	**0.005**
H × S	1	1.205	0.276	0.110	0.742	0.554	0.459	0.695	0.407	0.975	0.327	13.995	**<0.001**
N × S	1	0.141	0.709	0.371	0.544	0.377	0.541	0.000	0.982	0.975	0.327	2.054	0.156
H × N × S	1	0.201	0.655	0.030	0.863	0.666	0.417	0.005	0.941	0.001	0.980	1.923	0.170
Error	72												

Table B.2: Results from three factor analysis of variance (ANOVA) of sediment accumulation data. Data were rank-transformed. Significant results are highlighted in bold.

Effect	*df*	Inorganic sediment (g)		Organic sediment (g)		Organic sediment (%)	
		F	*P*	*F*	*P*	*F*	*P*
Herbivory (H)	1	53.595	**<0.001**	24.281	**<0.001**	64.439	**<0.001**
Nutrients (N)	1	0.019	0.892	0.075	0.786	0.000	0.999
Site (S)	1	32.249	**<0.001**	17.310	**<0.001**	29.571	**<0.001**
H × N	1	1.353	0.249	0.211	0.648	2.504	0.118
H × S	1	0.243	0.624	0.662	0.419	7.160	**0.009**
N × S	1	0.032	0.859	0.528	0.470	0.902	0.346
H × N × S	1	0.270	0.605	0.161	0.690	0.001	0.977
Error	72						

Table B.3: Results from three factor analysis of variance (ANOVA) of coral growth data. Data were rank-transformed. Significant results are highlighted in bold.

Effect	*df*	*Porites cylindrica* growth (%)		*df*	*Acropora millepora* growth (%)	
		F	*P*		*F*	*P*
Herbivory (H)	1	0.044	0.834	1	3.287	0.075
Nutrients (N)	1	0.008	0.930	1	0.540	0.466
Site (S)	1	13.512	**<0.001**	1	14.896	**<0.001**
H × N	1	0.198	0.658	1	0.931	0.339
H × S	1	2.101	0.152	1	0.061	0.806
N × S	1	0.205	0.652	1	0.653	0.422
H × N × S	1	1.534	0.220	1	0.205	0.653
Error	67			54		